U.S. DEPARTMENT OF COMMERCE
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PB-270 694

Chemical/Biological Implications of Using Chlorine and Ozone for Disinfection

Minnesota Univ -Duluth Dept of Chemistry

Prepared for

Environmental Research Lab -Duluth, Minn

Jun 77

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)	
1. REPORT NO. EPA-600/1-77-066	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE Chemical/Biological Implications of Using Chlorine and Ozone for Disinfection		5. REPORT DATE June 1977 Issuing date 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Robert M. Carlson and Ronald Caple		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Chemistry University of Minnesota-Duluth Duluth, Minnesota 55812		10. PROGRAM ELEMENT NO. 1BA608 11. CONTRACT/GRANT NO. Grant R-800679
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory-Duluth, MN Office of Research and Development U.S. Environmental Protection Agency Duluth, Minnesota 55804		13. TYPE OF REPORT AND PERIOD COVERED Final Project 1972-76 14. SPONSORING AGENCY CODE EPA/600/05
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

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The effects of chlorination on biological oxygen demand (BOD) were examined by comparing the BOD requirements of a sample containing a given parent system vs that of its chlorinated progeny. The results indicate that the chlorinated material is generally degraded less than the parent and that the lowered BOD values appear, at least for phenols, to be associated with the increased toxicity of the chlorinated material to the degrading organism.

17. KEY WORDS AND DOCUMENT ANALYSIS			
a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Chlorination Ozonization Disinfection Water Treatment Bioassay Toxicity Contaminants	Chemical analysis Chemical composition Biochemical oxygen demand Chemical reactions	Chemical structure-toxicity correlations	06/F 06/T 07/C
18. DISTRIBUTION STATEMENT RELEASE TO THE PUBLIC		19. SECURITY CLASS (This Report) UNCLASSIFIED 20. SECURITY CLASS (This page) UNCLASSIFIED	21. NO. OF PAGES 99 22. PRICE PC A05 / MF A01

EPA Form 2220-1 (9-73)

U.S. GOVERNMENT PRINTING OFFICE: 1977-757-056/6553 Region No. 5-11

PB 270 694

NATIONAL TECHNICAL
INFORMATION SERVICE

[redacted]

[redacted] reports [redacted] Office of Research and Development, U.S. EPA [redacted] [redacted] [redacted]

EPA-600/3-77-066
June 1977

CHEMICAL/BIOLOGICAL IMPLICATIONS OF USING CHLORINE AND OZONE FOR DISINFECTION

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FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota develops methods, conducts laboratory and field studies, and extrapolates research findings

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man.

This report provides a capability to not only predict more accurately the types of organic materials anticipated from chlorine and ozone disinfection but also the biological impact (i.e., toxicity and degradability) of these "second-order" products.

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ABSTRACT

Chlorine has been found to react readily in an aqueous medium with a variety of materials (α-terpineol, oleic acid, abietic acid, cholesterol, etc.) known to be present in waste waters subjected to chlorine renovation processes. The product distribution varies with pH, with a generally higher level of chlorine incorporation occurring with decreasing pH. The individual components (chlorides, chlorohydrins, epoxides, etc.) are predominantly those expected on the basis of the initial ionic attack of a positive chlorine species. Examples of aromatic compounds that were examined followed the generally accepted rules for substituent effects such that the introduction of a chlorine reduced the susceptibility to further reaction and that phenols were the most vulnerable to electrophilic attack. The aqueous chlorination of biphenyl produced predominantly the mono- and dichloro species.

Aqueous ozonation of α-terpineol and oleic acid produced olefinic cleavage products (i.e., aldehydes, ketones, and carboxylic acids) typical of those found previously in non-aqueous systems.

In a test to examine the biological effects associated with chlorine incorporation, the toxicity of a given system to Daphnia magna generally increased with chlorine content. The excellent correlation of these results in a Hansch structure-activity analysis with phenols suggests wide applicability of this technique for predicting the effects of a given chemical on the environment. In this correlation the dominant physical parameter was the partition coefficient, which, in turn, led to the development of a rapid method for determining the partition coefficient by using the retention properties of the compound in question on a "reverse-phase" high pressure liquid chromatography column. In addition, as part of this overall evaluation of the hydrophilicity of phenols, synthetic procedures were developed for the preparation of chlorophenylphenols (i.e., the presumed metabolic products of PCB's).

The effects of chlorination on biological oxygen demand (BOD) were examined by comparing the BOD requirements of a sample containing a given parent system vs. that of its chlorinated progeny. The results indicate that the chlorinated material is generally degraded less than the parent and that the lowered BOD values appear, at least for phenols, to be associated with the increased toxicity of the chlorinated material to the degrading species.

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ACKNOWLEDGMENTS

The authors wish to acknowledge the valuable contributions to the program that were made by the following individuals:

Research Fellows	Dr. Ted Garbacik
	Dr. Richard Hallchar
	Dr. Herbert Kopperman
	Dr. Gerald Putz
	Sr. Agatha Riehl, Ph.D.
Graduate Students	Mr. Ned Hillerin
Undergraduates	Mr. Robert E. Carlson
	Ms. Deborah Massey Jacobson
	Ms. Kathleen Mielke
	Mr. John Nelson
	Mr. Robert Schmitt
Technician	Mr. Steve Davis
Research Chemist, Lake Superior Basin Studies Program	Mr. Duane Long

We would also like to express our appreciation to Dr. Gary Glass, Dr. Kenneth Biesinger and the staff of the Environmental Research Laboratory, Duluth, for providing the necessary space and advice to obtain a meaningful bioassay. The computer programs, which are an integral part of the bioassay and structure-activity analysis, were developed with the invaluable assistance of Dr. Donald Harriss, Department of Chemistry, University of Minnesota, Duluth.

SECTION 1

CONCLUSIONS

Chlorine is readily incorporated into a variety of organic materials known to be present in water subjected to chlorine-renovation procedures. The observed products can be predicted on the basis of commonly used mechanistic considerations. In addition, these second-generation materials generally have increased toxicity or possess structural features (i.e., an epoxide or reactive alkyl halide) that have been associated with carcinogenicity.

The major products formed in the aqueous chlorination of α-terpineol(1) apparently can be accounted for by two trans Markovnikov adducts of the elements of hypochlorous acid. The pH profile of this reaction and the formation of other minor products can be related to the major trans adducts by secondary reactions that appear to follow the expected steric, torsional, and electronic demands of the menthane derivatives. These results will be useful not only in cataloging toxicity information, but also perhaps in providing a mechanistic basis for a study of related chlorinated terpenoids.

Cholesterol and oleic acid provide a much less complex mixture of products; the chlorohydrins predominate in both cases. The isolation of cholesterol epoxide further illustrates the possibility of obtaining this type of material during dilute aqueous chlorination.

The resin acids (abietic and dehydroabietic acid) give a variety of compounds upon chlorination that can be interrelated through various synthetic operations. An important aspect of this portion of the study was the observation of an apparent "free-radical" chlorination at the benzilic position on dehydroabietic acid.

The aqueous ozonation studies confirm that mechanistic considerations developed in non-aqueous cases can be applied to the prediction of products from ozone addition to dilute solutions of unsaturated organics in water.

Hansch "structure-activity" relationships are useful in predicting the potential impact of various organic materials on aquatic organisms. The dominant feature in the observed toxicity of phenols to Daphnia magna was the lipophilic nature of the compound as represented by the partition coefficient. The partition coefficient of a compound has been shown as part of this overall study to be readily obtained from its retention properties on a "reverse-phase" HPLC column. This ability to rapidly obtain a measure of a compound's hydrophilicity has implications for predicting properties other than toxicity (i.e., bioaccumulation).

The 500 test should not be applied to situations involving chlorinated organics, because apparent BOD reduction is derived from an enhanced resistance to degradation or increased toxicity to the microbial population used in the test, or both. In addition, the present method of comparing a parent compound and its chlorinated progeny appears to be a more sensitive probe of the relative degradative potential, as previous studies have not accounted for the small fraction of chlorinated organics in comparison with the unchlorinated organics in the renovated water.

SECTION 8

RECOMMENDATIONS

1. The detailed information on the products obtained from the chlorination of α-terpineol (or one of the other systems studied) should be applied to a situation (e.g., a pulp mill effluent) where the possible products can be most readily observed.

2. The aqueous chemistry of chloramines with very reactive organics such as phenols should be examined.

3. The cause of an observed BOD reduction, i.e., whether the organics show a resistance to degradation, should be determined for several different systems. This could be done by quantitatively examining the chlorinated organic before and after the test period.

4. The success in rapidly obtaining information on hydrophilicity by HPLC retention times and the demonstrated importance of this parameter in predicting toxicity should be applied to other areas. For example, there should be a gross HPLC analysis of complex effluents to determine which fraction would have the maximum probability of exhibiting toxicity or bioaccumulation effects.

5. Other methods of wastewater renovation (in particular, with ozone and ClO_2) should be examined chemically and biologically to ascertain if these methods represent a suitable alternative to chlorination.

6. The chemical and biological implications of applying chemical disinfectants to waters containing polynuclear aromatic hydrocarbons (PAH) should be investigated.

SECTION 3

INTRODUCTION

Chlorination is the predominant technique used for water renovation and disinfection. The process has been applied to wastewater treatment problems, to the disinfection of drinking water and the preservation of acceptable water quality through distribution systems, to the solubilization of sludge (a "superchlorination" process involving large doses of chlorine), to the maintenance of hygienic conditions in closed swimming areas, and to the reduction of algal and bacterial growth in cooling towers.[1-5] The development of the technology for the effective application of chlorine has been considered largely responsible for saving thousands of lives that could have been lost through contracting any of several possible water-borne diseases. In short, chlorination has developed into what has been referred to as, "the most valuable and versatile tool available to the water chemist."[1]

The possible reaction of chlorine with materials present in the treated water has long been recognized,[6] mainly because of the very practical necessity for using more chlorine than was anticipated to meet given standards of turbidity, BOD,[7] or fecal coliform. Environmentally, chlorine and chloramines (as reaction products of ammonia, amino acids, or other amines with chlorine) are considered deleterious,[7,8] and considerable effort has been directed toward their removal by such processes as reduction (e.g., SO_2) or by adsorption-decomposition (activated charcoal), with the result that documented examples of incorporation of carbon-bound chlorine under conditions used in water treatment have been quite limited. Early chemical investigations were only initiated when the chlorination process generated problems of taste and odor.[9-11]

The addition of chlorine to water results in the rapid hydrolysis to hypochlorous and hydrochloric acids. Moreover, considering the k_2 of hypochlorous acid, the active chlorine species will be essentially all hypochlorous acid or all hypochlorite in changing from a pH of 5 to a pH of 9.[1,5]

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$$

$$k_1 = 3.94 \times 10^{-4} \text{ at } 20\text{-}25^\circ$$

$$HOCl \rightleftharpoons H^+ + OCl^-$$

$$k_2 = 3.2 \times 10^{-8} \text{ at } 20\text{-}25^\circ$$

In the presence of ammonia a very rapid reaction occurs to generate chloramine. The subsequent conversion to di- and trichloramine occurs at a slower rate. This facile generation of chloramine thereby represents an effective competitive process to the formation of "second-order" chlororganics where a water sample contains substantial amounts of ammonia.[5]

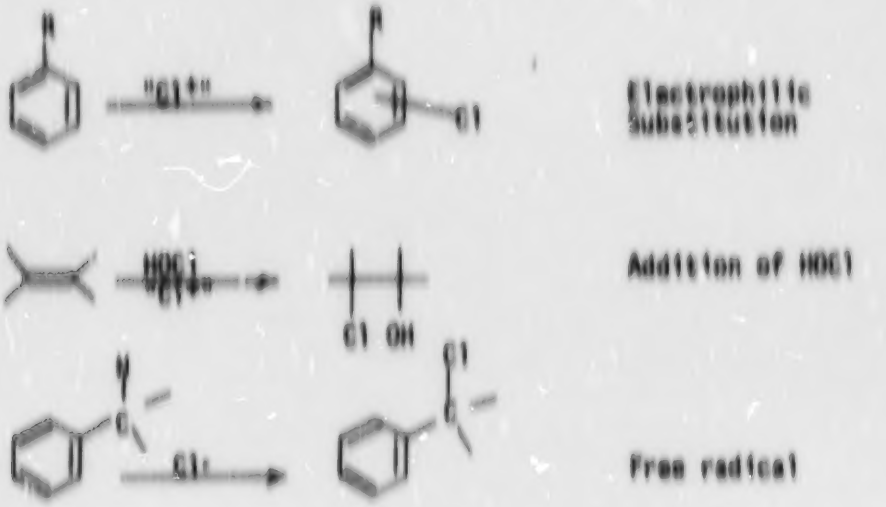
$$NH_3 + HOCl \rightleftharpoons NH_2Cl + H_2O$$
$$k_f = 8.6 \times 10^9$$

Recent determination of the organic content of water samples that are typically subjected to aqueous disinfection processes has shown that many compound types are present that could incorporate carbon-bound chlorine.[9-14] These types include aromatic compounds (i.e., phenols, amines, etc.), heterocycles, terpenes, sterols, resin acids, and unsaturated fatty acids. These studies not only indicate the complexity of the trace organics present in water, but, coupled with some fundamental organic chemical principles, give some insight into the possible "second-order" organic material that can be anticipated from any further chemical transformations. The presence of substantial amounts of "second-order" chlorinated pollutants arising from water-renovation processes that are not subject to reductive "dechlorination" has only recently gained attention because of the isolation of chlorinated materials from supplies of potable water.[15] Of equal scientific significance is the recent work by Jolley in which a combination of radioactive tracer techniques and high pressure ion-exchange chromatography demonstrated the presence of over 50 "new" organochlorine species from a wastewater chlorination process.[16] The magnitude of the observed % chlorine uptake as carbon-bound chlorine observed by Jolley can best be illustrated by a specific example. Thus, at an average flow of 43.6 MGD of municipal and industrial waste into the Western Lake Superior Sanitary District (WLSSD, Duluth, Minnesota, 100,600 population) and the use of 28 lbs. of Cl_2/MG, the waste-treatment facility will discharge 21,900 lbs. of chlororganics into Lake Superior during the course of a single year. This figure of nearly 11 tons of yearly discharge is conservative in that average flows were considered and Jolley was only examining "soluble" organic material, thereby eliminating from consideration the various types of polymeric material (humic acids, polypeptides, etc.) and volatiles (chloroform, etc.).

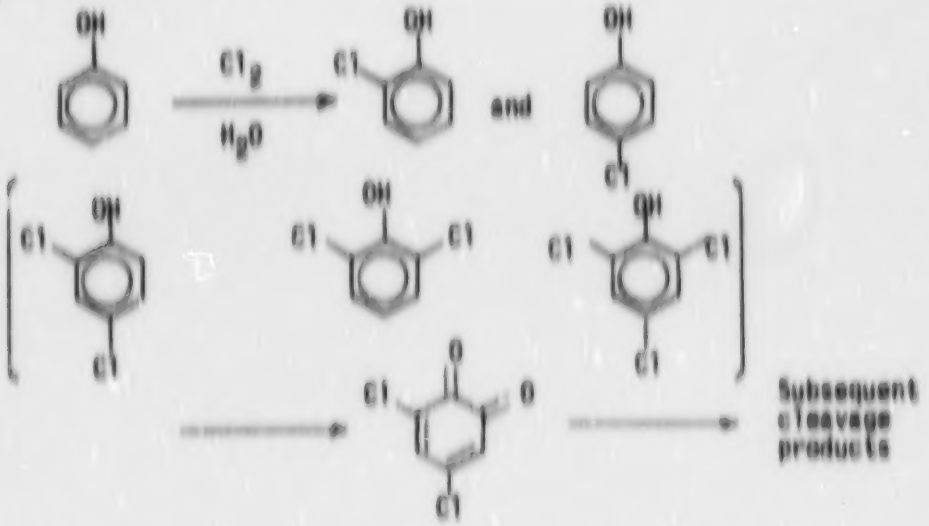
Average flow of municipal and industrial waste (WLSSD)	= 43.6 MGD
28 lbs of Cl_2/MGD	= 1,222 lbs. Cl_2/day
1% chlorine incorporated	= 12 lbs.
Average molecular weight of "new" chlororganic = 175	= 60 lbs chlororganics produced/day
Yearly discharge (X 365)	= 21,900 lbs./year

An a priori consideration of mechanistic organic chlorine chemistry suggests that the anticipated chlorine-containing organic products will be derived either from the attack of an electrophilic chlorine species (often represented by Cl^+) or an aromatic,[17] an olefinic, or other nucleophilic portion of the molecule or by a free radical process.[18] The former process will generate products such as halogenated aromatic systems (electrophilic aromatic substitution) and olefin-derived compounds such as chlorohydrins (addition reactions) and will proceed at a rate proportional to the availability of the electrons in the substrate. The radical process, although less likely in the polar aqueous medium, will be most readily observed from

those compounds that can provide a stable radical intermediate (e.g., benzilic halogenation).

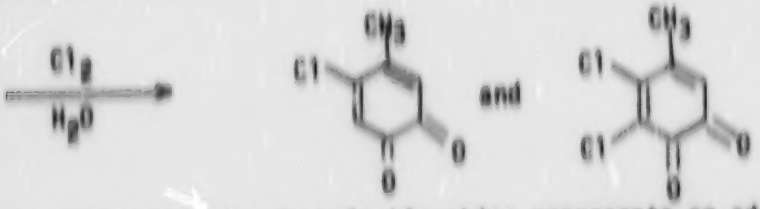


Aqueous electrophilic aromatic substitution processes have been thoroughly documented for phenols where chlorine can readily be incorporated throughout a wide range of pH values because of the reactive nature of the phenolic ring system. In the case of phenol itself, chlorination proceeds through ortho and para-chlorophenol to the higher di- and tri-chlorinated isomers (2,4- and 2,6-dichlorophenol and 2,4,6-trichlorophenol[5-7]), and the rate of chlorine incorporation decreases with increasing chlorine content. Further reactions consist of oxidation to the chloro-orthoquinone and subsequent cleavage rather than incorporation of additional chlorine atoms.[8]

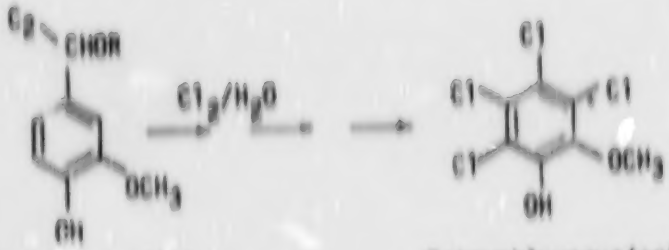


In strongly alkaline media, hypochlorite also results in degradation of the phenolic ring system. The products are of various types, including a series of interesting ring-contracted materials (e.g., cyclopentane-carboxylic acid derivatives) related to some known antibiotic systems.19

Cresols have been found to provide stable chloro-o-benzoquinones. This type of product has been shown to be a component of a bleached kraft mill effluent toxic to salmon.20,21



The delignification in pulp bleaching represents an additional example of phenolic chlorination chemistry. The incorporation of chlorine into the aromatic nucleus occurs not only by direct introduction, but also by an alternate process that can be referred to as electrophilic replacement.22 Other phenolic materials would be expected to react in a similar fashion to the examples cited.

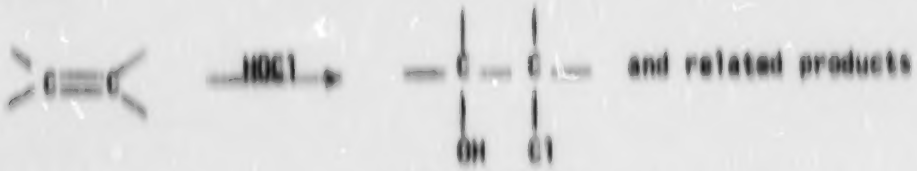


R = H or alkyl

Tetrachloroguaiacol

Polynuclear aromatic hydrocarbons (PAH) represent another ubiquitous compound type that has been shown to incorporate chlorine into the aromatic nucleus. For example, in a recent study of the aqueous chlorination of the water-soluble portion of diesel fuel a number of chloronaphthalenes were observed.23

An olefin represents an additional example of a site subject to electrophilic attack. In the case of the isolated olefin without hetero atom substituents the major products of aqueous chlorination are chlorohydrins, although 1,2-dichloro compounds, epoxides, and other compounds are also possible. Historically, although some of the oldest known addition studies dealt with electrophilic additions to monocyclic terpenes,[2] aqueous chlorinations often produced prohibitively complex mixtures that were unsuitable for any product or mechanistic study.[2a] Other olefinic compounds known to be present in water include unsaturated fatty acids (oleic, linolenic, etc.), cholesterol, resin acids, steroids, and other miscellaneous natural and industrial compounds.[3,4,14] The α-halogenation of carbonyl systems also belongs to this type of reaction when an enol is considered as a reactive intermediate.



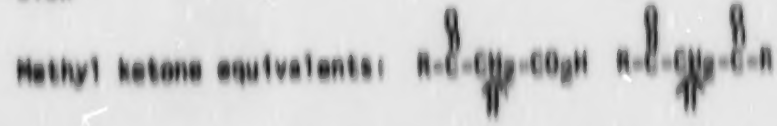
Chlorohydrin

Enol

Aromatic heterocycles (i.e., purines and pyrimidines) that are derived from the breakdown of nucleic acids have been observed by Jolley to incorporate chlorine. In that study 6-chlorouracil, 8-chlorocaffeine, 8-chlorouridine, and 8-chloroguanine and 8-chloroxanthine were present.

The halogenation products of carbonyl compounds vary with pH, but in the case of the haloform reaction (basic conditions, methyl ketones) the initial halogenation represents the slowest step. Potential enolic systems can be found in compounds possessing a carbonyl or functional group (e.g., -OH, -NH_2) that could represent a carbonyl precursor.

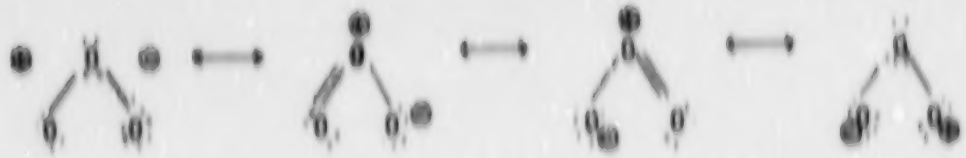
The ultimate products from chlorination of a methyl ketone will be chloroform and the carboxylic acid salt. Although it is generally considered that only methyl ketones can ultimately generate haloforms, there is considerable evidence that humic material is the source of the chloroform derived from chlorination of drinking water[5] and that a methyl ketone equivalent such as a β-keto acid, a β-keto aldehyde, or a β-diketone might represent the requisite reactive methyl ketone equivalent.

Methyl ketone equivalents: 

It is apparent that stable chlororganics may be introduced into aquatic systems in a host of ways. Adding to the overall concern about the environmental implications of such a phenomenon is the ever-increasing amount of recycled water that is being used and the possibility that these "second-order" organo-chlorine molecules might have some of the negative properties of their "primary" pollutant counterparts.[26,27] The possible environmental hazards associated with aqueous chlorination therefore strongly suggest that viable alternatives should be sought and evaluated.

Ozone represents one potential alternative to the use of chlorine in water-quality control in spite of the additional cost and the inability to maintain a residual. These deficiencies in the character of ozone are frequently offset by its reported superior qualities in removing taste, odor, and color and by its outstanding disinfecting capability.[28]

Ozone is a very reactive species with an oxidation potential second only to fluorine. Ozone is diamagnetic and, unlike oxygen, one should expect the initial attack of ozone to be non-free-radical, with ozone acting as an electrophile or as a 1,3-dipole, or both.



Information is limited, however, on the aqueous chemistry of ozonation, although kinetic studies have shown that the mechanism of ozonation apparently does not vary with solvent polarity.[29,30] Fortunately, therefore, much of the initial work already done with organic solvents can be applied to aqueous systems.

Ozone decomposes in water, and the sole decomposition product is oxygen.[25,26] Although the mechanism of decomposition is not agreed upon, the rate of this reaction increases with an increase in pH or an increase in salt concentration.[27] Most of the proposed ozone decomposition mechanisms are free radical[25,26], but the rate of aqueous decomposition is slow

with respect to ozonolysis[28] and for this reason the free-radical contribution by the decomposition intermediates is generally ignored.

"Among the organic groupings which can be oxidized by ozone are olefinic and acetylenic carbon-carbon multiple bonds, aromatic, carbocyclic and heterocyclic molecules; carbon-nitrogen and similar unsaturated groupings; nucleophilic molecules such as amines, sulfides, sulfoxides, phosphines, phosphites, arsines, selenides, etc.; carbon-hydrogen bonds in alcohols, ethers, aldehydes, amines, hydrocarbons, etc.; silicon-carbon, silicon-silicon, and silicon-hydrogen bonds; and carbon-metal bonds of various types."[30]

$\geq C{=}\ddot{N}-$; $:NR_3$; $R_2\ddot{S}:$; $R_2\ddot{S}{=}\ddot{O}$; $:PR_3$; $:P(OR)_3$;

$:AsR_3$; $R_2\ddot{S}e:$; H-C$\leq$ as in R_3C-H, $R-\overset{\overset{O}{\|}}{C}-H$, $R_2N-\overset{\overset{H}{|}}{C}R_2$,

$R-S-\overset{\overset{H}{|}}{C}R_2$, $R-O-\overset{\overset{H}{|}}{C}R_2$, etc.; $\geq Si-H$; $\geq Si-C\leq$;

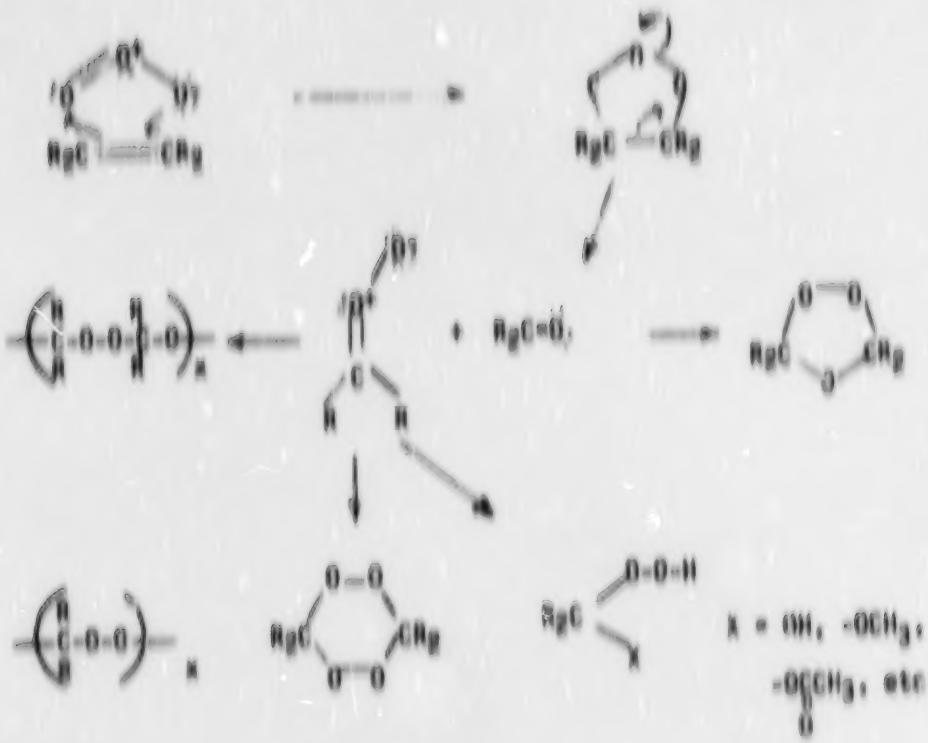
$\geq Si-Si\leq$; $\geq C-Mg-$, $\geq C-Hg-$, etc.

"Carbon-carbon double bonds are usually the most reactive in the above systems toward ozone, but nucleophiles such as arsines and selenides, as well as certain carbon-nitrogen double bonds, are nearly as reactive, and, in some cases, are more reactive. Carbon-hydrogen and silicon-hydrogen, etc., bonds are usually the least reactive of the preceding groupings present in the material being ozonized."[30]

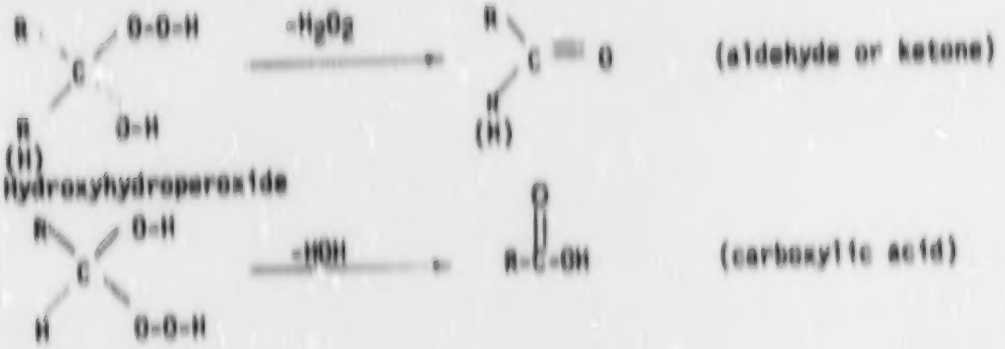
Alkenes represent the most reactive of a number of organic systems that are vulnerable to ozone attack. Criegee has proposed a three-step mechanism which explains the major products that are formed from alkene ozonolysis, and although other mechanisms have been proposed,[30] a recent and more comprehensive version of his original proposal is now generally accepted.[31-33]

"In aqueous media, the peroxidic ozonolysis product should be a hydroxy hydroperoxide. Although only a few studies of ozonolysis have been made in water media [34-38], the results indicate that the hydroxy hydroperoxide is fairly easily decomposed to an aldehyde (or ketone) or to a carboxylic acid, as shown below, although heat may sometimes be necessary to effect the transformation. Since aldehydes can also be converted to carboxylic acids by ozone (see later discussion) the end products of these reactions would be further oxidized by ozone only slowly and with great difficulty."[30]

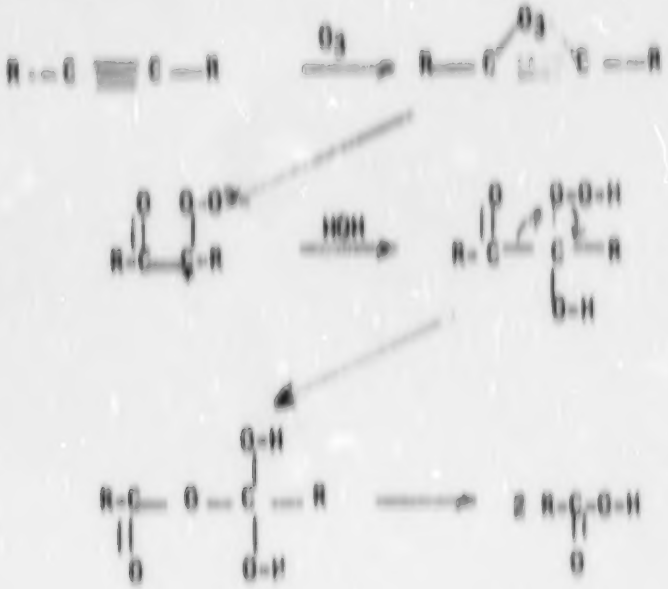
Revised Criegee Ozonolysis Mechanism



"With certain olefins, having two bulky substitutents at the carbon, or three at both carbons of the double bond, oxide formation competes with ozonolysis. These reactions appear to involve a purely electrophilic ozone attack, followed by loss of molecular oxygen, and occur both in polar (e.g., water) and non-polar solvents."[30]



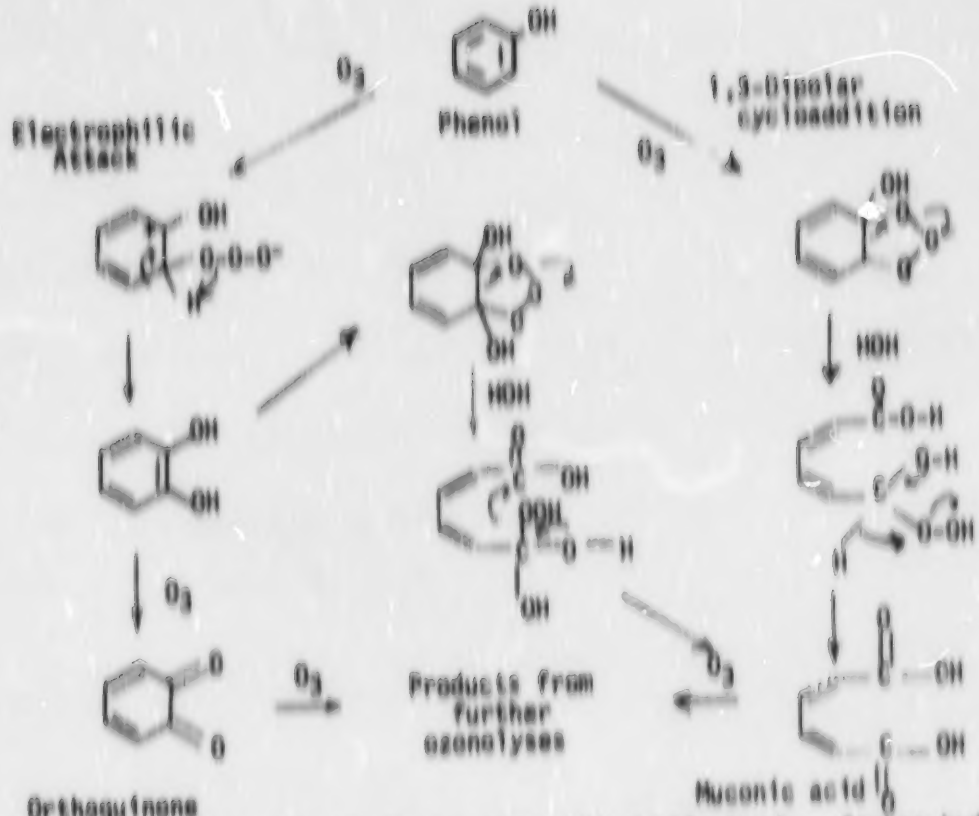
"Acetylenic compounds undergo ozonolysis at the triple bond. The Criegee mechanism appears to apply, although few mechanistic studies have been made. Based on this mechanism as shown below, the peroxidic intermediate should be that shown, and the final product should be a carboxylic acid."[30]



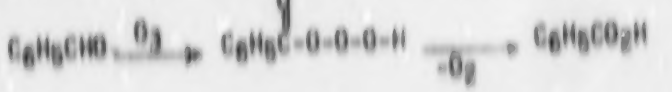
"The ozonation of aromatic compounds appears to involve both ozonolysis at the most reactive aromatic bond, and electrophilic ozone attack at individual carbon atoms.[30] In regard to ease of attack, the unsubstituted benzene ring is much less reactive toward ozone than in an olefinic double bond."[30,39] The effect of substituents on the rate of ozonolysis is similar to other electrophilic additions where alkyl, aryl, oxygen, etc. substituents facilitate the reaction, whereas groups such as nitro, carboxyl, halogen, and sulfonic acid substituents slow the reaction. The cleavage products of benzene ring systems are the expected glyoxals (R-C(=O)-C(=O)-H) and glyoxalic acids (R-C(=O)-CO$_2$H).

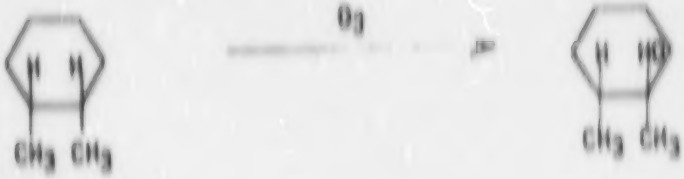
The only aromatic system that has been studied to any extent in aqueous media is phenol and its homologs. A study of ozonation of phenol itself suggests the intermediacy of several intermediates including catechol, the orthoquinone, and muconic acid.[39]

Other organic compound types would also be expected to react with ozone. Polynuclear aromatics such as anthracene, naphthalene, and phenanthrene are more reactive than benzene derivatives, but less reactive than olefins. Amines, sulfides, and selenides are oxidized to their corresponding oxides.



and in some cases ozonation is faster than with double bonds. Carbon-hydrogen bonds can also be oxidized by ozone through the hydrotrioxide intermediate.[29]





Stable hydroperoxide derivatives can be formed during the ozonation process as illustrated by Criegee's study of the ozonolysis of a cyclic olefin in the presence of ammonia[30] and in the ozonolysis of a cyclic sulfone.[29]

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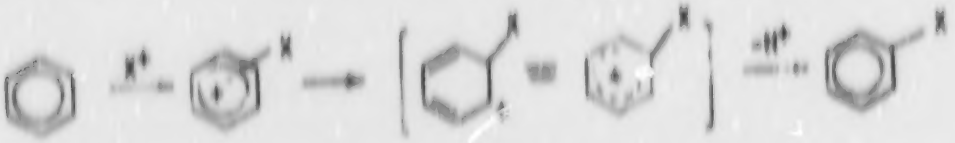
SECTION 4

CHEMICAL STUDIES

AROMATIC CHLORINATION

Results and Discussion[1]

The introduction of chlorine into an aromatic system represents an example of electrophilic aromatic substitution, for which there is a single unifying mechanism with respect to substrate.[2,3,4]



When the aromatic system is monosubstituted, the reaction may be faster or slower than with benzene itself, and the aromatic electrophilic substitution process will result in a product that is ortho (1,2-disubstitution), meta (1,3-disubstituted), or para (1,4-disubstituted).[5]

Activating groups are those which increase the reaction rate, whereas deactivating groups are those which decrease the reaction rate. All groups that give predominantly meta products are deactivating; most of the ortho-para-directing substituents are activating. The notable exceptions are the halogens. Although a halogen is deactivating (i.e., less reactive than without the halogen) the products of subsequent electrophilic substitution will be predominantly ortho-para.

Monosubstituted aromatics were exposed to low concentrations (7.0×10^{-4} M) chlorine for twenty minutes (Table 1). The extent of chlorine incorporation followed well-recognized trends, i.e., aromatics containing "activating" groups such as hydroxyl, ether, amine derivatives, or alkyl underwent electrophilic aromatic substitution faster than those containing "deactivating" groups such as nitro, chloro, nitrile and carbonyl. Phenol is unique in its ready chlorination at high pH values; although the chlorinating agent is hypochlorite, the substrate is the very reactive phenolate anion.

The aqueous chlorination process was analysed in detail by using biphenyl as the aromatic species. Biphenyl was chosen for study because the methodology for isomer recognition is available and because considerable interest has recently centered on the possible production of polychlorinated biphenyls (PCBs) at waste-treatment facilities known to receive biphenyl.[6] The results shown in Table 2 confirm the possibility of chlorine incorporation into the biphenyl nucleus under various aqueous conditions. The extent of chlorobiphenyl production was dependent on pH. Above pH 4 extended reaction times were required for extensive chlorine incorporation. Substantial amounts of higher chlorinated isomers occur at increased chlorine concentrations, an important observation if potential problems associated with "superchlorination" for such purposes as sludge solubilization are being considered.

Experimental

Procedure for Aromatics Study (Table 1) --

All reactions were carried out in a 500-ml volumetric flask. To each flask was added 12.72 ml of the 3.84-mg/ml NaOCl solution, HCl (for pH adjustment), and the test solution. The reaction temperature was maintained at 25° ± 0.2°C by using a constant temperature bath. A 20-ml aliquot was taken at twenty minutes and added immediately to a flask containing 15 ml of 0.00543N $Na_2S_2O_3$ solution, 5 ml of HOAc, 2 ml of KI (50% aqueous) and 1-2 ml of freshly prepared starch solution. This was titrated to a faint purple color with 0.096 mg/ml of NaOCl solution.

Procedure for Biphenyl Study (Table 2) --

To five of the bottles containing 100 ml of distilled water and saturated biphenyl, 100 ppm of $Ca(OCl)_2$ were added. The remaining five bottles, containing water and biphenyl only, served as controls. The pH of the final solutions was approximately 6.5. Samples were extracted at intervals of one day, one from each concentration of $Ca(OCl)_2$ and a control, resulting in exposure periods of 1, 2, 3, 4, and 5 days. The entire contents of a bottle were transferred to a 250-ml separatory funnel and extracted three times with 25 ml of hexane. The bottles were rinsed with the first 25-ml aliquot. The combined hexane extracts were filtered through anhydrous sodium sulfate and concentrated in a Kunderna-Danish evaporator concentrator. The hexane concentrates were analysed with electron capture gas chromatography (EC/GC) under the following conditions.

Analysis conditions (samples 1-9) -- Instrument: Hewlett-Packard 5753A (Ni-63 Electron Capture Detector). Column: 2M x 1/4" glass-4% XE-60 silicone on 80/100 mesh HP Chromosorb W. Injection temperature: 200°C. Column temperature: 130°C isothermal. Detector temperature: 250°C. Carrier: helium @ 60 ml/min. Purge: 10% methane/argon @ 120 ml/min. Pulse interval: 50 usec.

Analysis conditions (samples 10-15) -- Instrument: Tracor Model 550 Gas Chromatograph (tritium-electron capture detector). Column: 6' x 4mm ID glass column -- 3% OV_7 on 80/100 HP chromosorb W. Injection temperature: 200°C. Carrier: nitrogen @ 70 ml/min. Purge: nitrogen @ 30 ml/min.

TABLE 1. PERCENTAGE CHLORINE UPTAKE BY VARIOUS AROMATICS AT THREE pH VALUES

	pH		
	3	7	10.1
(9.5 ± 0.5 × 10⁻⁴ M)	% Cl (uptake)	% Cl	% Cl
Phenol	97.8 ± 0.1	97.6 ± 0.1	97.6 ± 0.1
Anisole	80.7 ± 0.2	11.4 ± 0.4	0.8 ± 0.1
Acetanilide	55.3 ± 0.5	3.4 ± 0.2	—
Toluene	11.1 ± 0.1	2.9 ± 0.4	—
Benzyl alcohol	0.3 ± 0.3	—	—
Benzonitrile	0.1 ± 0.2	—	—
Nitrobenzene	1.8 ± 0.1	—	—
Chlorobenzene	1.8 ± 0.1	—	—
Methyl benzoate	1.8 ± 0.2	—	—
Benzene	1.5 ± 0.1	—	—

Chlorine (7.8 × 10⁻⁴ M), 20 min, 25°C.

TABLE 2. CHLORINE INCORPORATION INTO THE DIPHENYL NUCLEUS UNDER VARIOUS AQUEOUS CONDITIONS

Chlorine source	pH	Chlorine, ppm	Reaction time, hr	Reaction, %	Ppb of chlorinated product				
					3	5.4	8.9	9.8 9.4	4.0
[illegible]	5.5	100	24	0.4	15	10			
[illegible]	5.5	100	48	1.0	32	27			
[illegible]	5.5	100	72	1.7	55	42			
[illegible]	5.5	100	120	2.5	60	50			
[illegible]	5.5	10	120	0.004	0.1	0.15			
[illegible]	5.5	20	120	0.02	0.44	0.61			
[illegible]	5.5	35	120	0.04	1.4	1.2			
[illegible]	5.5	50	120	0.15	4.0	4.5			
[illegible]	5.5	100	120	0.37	8.8	7.4			
Cl_2	2.1	10.9	0.25	2.2	120	10			20
Phenol	2.8	200	0.25	0.5	450	190	10	130	40
Cl_2	4.0	200	0.25	0.1	5–10				
Phenol	10.4	830	0.25	0.6	90				
Cl_2	2.2	1350	0.25	10	180	80	80	370	500
Phenol	7.0	2000	0.25	5.0	280	110		60	30

[illegible] monohydrate to be 0.0 mg/l.

Compound identification and calibration -- Chlorinated isomers were designated by comparison with pure materials (as determined by GC-MS). The percentage reaction is based on peak areas as compared to standards and on the experimentally determined solubility of biphenyl (6.0 mg/l).

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α-TERPINEOL (CHLORINATION)

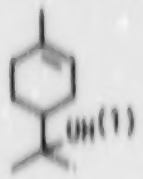
Results and Discussion

Historically, some of the oldest known addition studies dealt with electrophilic additions of halogens to monocyclic terpenes.[1] Not surprisingly, the reactions were somewhat restricted to conditions that produced manageable results (i.e., bromine, in a non-polar solvent). Furthermore, aqueous halogenations, especially aqueous chlorinations, often produced prohibitively complex mixtures of second-order chlororganics that were unsuitable for any subsequent product or mechanistic studies. However, the possibility of forming stable and toxic chlorinated derivatives from the widespread use of chlorine in disinfection, wood-pulp bleaching, and related processes[2,3,4] made imperative an examination of the aqueous chlorination chemistry of a common monocyclic terpene.

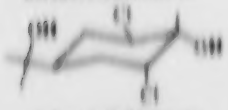
We have, therefore, interpreted in detail the aqueous chlorination chemistry of α-terpineol (p-menth-1-en-8-ol, 1), an excellent example of ubiquitous naturally occurring monocyclic terpene.[5-9] Although an earlier investigation of the interaction of α-terpineol (1) with sodium hypochlorite gave an unidentified mixture of products and was inconclusive,[10,11] we reapproached this problem with the aid of modern chromatographic techniques. Our ultimate goal was to provide a mechanistic interpretation[12] and a pH profile of the observed product distribution to aid in the prediction of products derived from related systems.

The aqueous chlorination of α-terpineol (1) was, therefore, examined over a pH range of about 2 to 10, the lower pH corresponding closely to a saturated chlorine solution and the higher pH to a sodium hypochlorite solution. Considerable effort was made to detect and identify every observable compound, as relative yields have less significance when one is concerned with bioaccumulation and toxicity. In the present analysis (LC and glc) products in the half-percent range could be reproducibly detected (Table 3).

TABLE 3. PRODUCTS FORMED IN THE AQUEOUS CHLORINATION OF α-TERPINEOL[a]



Dichloro Derivatives[b]



β,3 or 6-dichloro-cis-p-menthane-1,8-diol (6)

1,6-dichloro-p-menthane-8-ol (5)

α,3-dichloro-cis-p-menthane-1,8-diol (4)

β,3-dichloro-trans-p-menthane-1,8-diol (6)

6-chloro-cis-p-menthane-1,8-diol (8)

Monochloro Derivatives[b]

Non-chlorinated Products

p-menthane-α,1,2-epoxy-8-ol (7)

p-menthane-β,1,2-epoxy-8-ol (9)

1-endo-cineolylol (9)

[a] Not all of the products are formed at any given pH; see product distribution as function of pH in Table 4.

[b] Chlorine content established by mass spectral analysis and microanalysis.

[c] Acceptable names based on alternate p-menthane nomenclature are, for 4, 1,8-dihydroxy-neoisomenthyl chloride; for 5, 1,8-dihydroxyneomenthyl chloride; for 7, trans-p-menthane-1,2-epoxy-8-ol; for 8, cis-p-menthane-1,2-epoxy-8-ol.

The distribution of these products as a function of pH is given in Table 4. The distribution was similar from pH 2 to 6, and, therefore, only one set of values (at pH 2.2) is given for this range. Likewise, the results above pH 7.9 were similar; only the values for pH 10 are provided. The results are reproducible for a given set of experimental conditions, and the percentages represent all observable products. The percentages

agree with actual isolated yields, and apparently all compounds present at 0.5% or higher were detectable by analytical HPLC.

The chlorine addition reactions, whether starting with chlorine gas (for highly acidic reactions) or sodium hypochlorite solutions, were run with about two equivalents of available chlorine. This quantity is essentially the minimal quantity necessary to insure the complete reaction of α-terpineol (1). Reactions with a larger excess of chlorine, and reaction times beyond the disappearance of α-terpineol (1), led to only a very small and slow increase in the chlorine content of the chlorinated mixture, presumably via a free radical substitution or a dehydration and addition sequence, or both. Thus, the product distribution was quite insensitive to small changes in reaction conditions.

TABLE 4. PERCENTAGE DISTRIBUTION OF CHLORINATION PRODUCTS OF α-TERPINEOL (1) AT pH 2.2 and pH 10[a,b]

Compound[c]	pH 2.2	pH 10
1	0	0
2	9	0
3	2	0
4	29	42
5	53	0
6	4	19
7	1	39
8	1	0
9	1	0

[a] A LC analysis on a Corasil column of the ether extracts.

[b] The data are reproducible for a given set of experimental conditions and are believed to be accurate to ±1%. The percentages were obtained by integration of results on LC column and are not corrected for molar purposes.

[c] Toxicity: With the exception of 3, the compounds formed have a toxicity to Daphnia magna, LC_{100} (48 hrs.), similar to or even less than that of α-terpineol (120 ppm). The dichlorides 3 are an order of magnitude more toxic at about 15 ppm.

An examination of the major products formed at a pH of 2.2 and 10 revealed a self-consistent pattern. The major product under the acidic conditions was assigned to the trans-diaxial adduct 5 of hypochlorous acid. The configuration at C_5 is readily established by pmr, δH_5 = 4.09, $J_{5a,6a}$ =

$J_{5a,6a}$ = 3.9 Hz, but the assignment at C_1 is based on mechanistic grounds and conversion into the corresponding epoxide 7.

Not unexpectedly, the major products under basic reaction conditions were the α-epoxide 7 derived from the cyclization of the diaxial chlorohydrin 5 and the trans-diequatorial chlorohydrin 4.

The major products formed in the aqueous chlorination of α-terpineol (1) can, therefore, be accounted for by two trans Markovnikov adducts of the elements of hypochlorous acid. The pH profile of this reaction and the formation of other minor products can be related to the major trans adducts by secondary reactions that appear to follow the expected steric, torsional, and electronic demands of the menthane derivatives. These interactions are summarized in the flow diagram in Figure 1. These results will be useful not only in cataloging toxicity information, but will provide a mechanistic basis for a study of related chlorinated terpenoids.

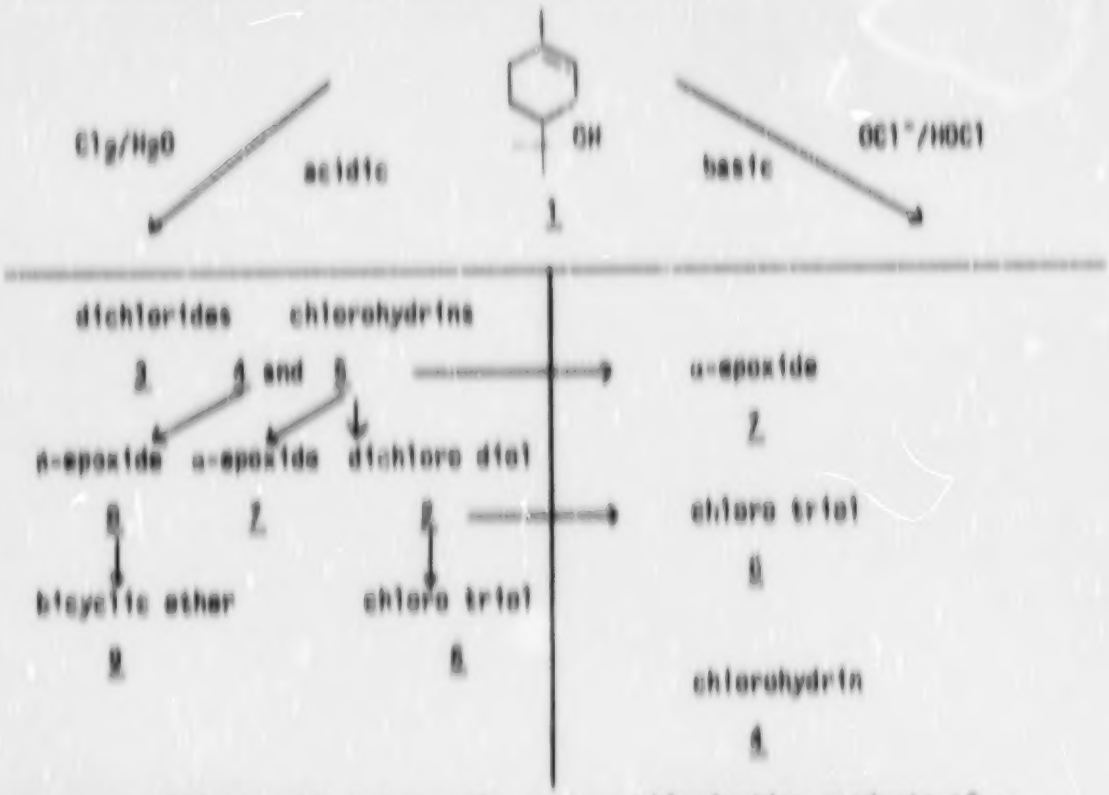


Figure 1. Relationship between the aqueous chlorination products of α-terpineol (1).

Experimental

Measurements of pH were recorded by a Corning Scientific Instruments Model 7 pH meter. Analytical vapor phase chromatography was performed on

a Tracor Model 550 (FID) detector). Preparative work was carried out on a Varian Autoprep Model A-700 (TC detector). Melting points were determined with a Thomas Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, New York. Nmr spectra were obtained with a Varian Associates Model A-600 Spectrometer; tetramethylsilane was used as an internal reference. Mass spectra were obtained on a Varian CH6 instrument and infrared on a Beckman IR 33. The liquid chromatographic monitoring of samples was performed on 1/8 x 2' Corasil II columns that were mounted in a Waters Associates ALC 202 (differential refractive index detector). Product resolution was accomplished in two solvent systems and their separations are summarized in Table 5.

TABLE 5. LIQUID CHROMATOGRAPHIC SEPARATIONS OF α-TERPINEOL CHLORINATION PRODUCTS

Polar		Non-Polar	
Solvent System:[a] 99% $CHCl_3$, 1% CH_3OH		Solvent System:[b] 50% C_6H_{14}/$CHCl_3$	
Compound	Elution Time (min)	Compound	Elution Time (min)
2	1.25	3	2.0
4	1.75	1	2.5
5	2.25	7	5
6	6.5	9	6
		8	7

[a] Corasil II column, 0.5 ml/min

[b] Corasil II column, 2.0 ml/min

Reagents --

The α-terpineol (1) used was obtained from Eastman Organic Chemicals and was purified by fractional distillation at reduced pressure. Sodium hypochlorite, 5%, was obtained from the J. T. Baker Chemical Co.

Preparative Chlorination of α-Terpineol (1) --

For the preparative formation of the product mixture, a high concentration chlorination of α-terpineol (1) was carried out by passing chlorine gas through a stirred mixture of α-terpineol (6 g) in 3 l. of water until the yellow color of chlorine persisted. The excess chlorine was allowed to escape by stirring overnight. The product mixture was removed by a continuous ether extraction, and, after drying over magnesium sulfate, the removal of the solvent left 6 g of an oily residue. The product distribution, as determined by analytical LC, was similar to the controlled reactions

used in the product distributions given in Table 4. Separation was accomplished by column chromatography on silica gel with an initial benzene elution and subsequent ether/benzene elutions, increasing the ether to benzene ratio until pure ether was the eluent. The separation was monitored by analytical LC.

β-2, α-6-Dichloro-cis-p-menthane-1, 8 diol (2) --
The column chromatography fraction corresponding to 2 was purified by recrystallization from chloroform, mp 138-139°; nmr (acetone-d_6), δ 1.18 (s, 3, CH_3), δ 1.60 (s, 3, CH_3), δ 2.95 (s, 1, OH), δ 4.10 (s, 1, OH), δ 4.10 (m, 1, H, W_h = 9 Hz). The mass spectral and ir characteristics are consistent with the dichlorodiol 2.

Analysis -- Calcd for $C_{10}H_{18}O_2Cl_2$: C, 49.80; H, 7.52.

Found: C, 49.76; H, 7.56.

1,2-Dichloro-p-menthane-8-ols (3) --
The mixture of dichlorides 3 corresponding to 3 was obtained independently by dissolving α-terpineol (0.31 g, 0.001 mol) in 14 ml carbon tetrachloride containg chlorine (0.071 g, 0.001 mol). The solvent was removed after 0.5 hr, and the crude product was subjected to column chromatography conditions corresponding to those applied to the products obtained from the aqueous chlorination. The resulting dichlorides had the expected mass spectral, ir, and nmr characteristics, and they corresponded to those for the product isolated from the aqueous preparative reaction.

α-2-Chloro-cis-p-menthane-1, 8-diol (4) --
As can be seen from Table 4, a basic heterogeneous chlorination of α-terpineol (1) provides the best conditions for obtaining the diequatorial chlorohydrin 4. In the preparative formation of 4, 10 g (0.065 mol) of α-terpineol was mixed with 0.5 l. of water. The mixture was stirred vigorously, and 150 ml of a 5% sodium hypochlorite solution was added. The pH was brought to about 8 by the dropwise addition of concentrated hydrochloric acid. Stirring was continued for 2 hrs, and then a solution of sodium sulfite was added to destroy excess chlorine. The aqueous mixture was extracted with ether in a continuous extraction apparatus. The combined ether extracts were dried over anhydrous magnesium sulfate, and the ether was removed under reduced pressure which yielded 10 g of a yellow oil. Crystals formed upon standing, and they were recrystallized from a 3:1 solution of chloroform: hexane; mp 118.5-119.5°, nmr ($DCCl_3$), δ 1.20 (s, 6, CH_3), δ 1.30 (s, 3, CH_3), δ 4.00 (dd, 1, H, $J_{2,3a}$ = 11.0 Hz and $J_{2,3e}$ = 4.5 Hz). The mass and ir spectra were in agreement with the monochloro diol structure.

Analysis -- Calcd for $C_{10}H_{19}O_2Cl$: C, 58.11; H, 9.26; Cl, 17.15.

Found: C, 58.13; H, 9.25; Cl, 17.31

β-2-Chloro-trans-p-menthane-1,8-diol (5) --
The peak corresponding to 5 in the preparative acetate aqueous chlorination of α-terpineol (1) was purified by recrystallization from chloroform,

mp 93-94°, nmr (acetone-d_6), δ 1.18 (s, 6, CH_3), δ 1.28 (s, 3, CH_3), δ 2.96 (m, 1, OH), δ 2.95 (s, 1, OH), δ 4.09 (m, 1, H_8), mass and ir spectra confirmatory.

Analysis -- Calcd for $C_{10}H_{19}O_2Cl$: C, 58.10; H, 9.26; Cl, 17.15.

Found: C, 57.95; H, 9.08; Cl, 16.86.

6-Chloro-cis-p-menthane-1,2,8-triol (6) --
This product can be isolated from either the acidic or basic aqueous chlorination. Purification was accomplished by recrystallization from acetone, mp 146-147°; nmr (acetone-d_6), δ 1.16 (s, 6, CH_3), δ 1.27 (s, 3, CH_3), δ 3.10 (m, 2, OH), δ 3.92 (m, 2, H_2 & H_6), δ 4.55 (d, 1, OH, J = 6 Hz); mass and ir spectra confirmatory. This triol can also be prepared by the hydrolysis of the dichloro diol 2. Thus when 2 was dissolved in 1 M hydrochloric acid and stirred at 25°C for 48 hrs, and worked up by ether extraction, the crystalline product obtained was identical in physical and spectral properties as well as mixed melting point with the triol 6.

Analysis -- Calcd for $C_{10}H_{19}O_3Cl$: C, 53.95; H, 8.60; Cl, 15.92.

Found: C, 53.93; H, 8.75; Cl, 15.81.

m-Chloroperoxybenzoic acid epoxidation of α-terpineol (1) --
The α-epoxide 7 and bicyclic ether 8 are best synthesized by direct epoxidation of α-terpineol (1). In a one-liter flask, fitted with a dropping funnel and a $CaCl_2$-filled drying tube, were placed 250 ml of methylene chloride and 8 g of 80% (0.04 mol) m-chloroperbenzoic acid (Aldrich Chemical Co.). This solution was cooled in an ice-water bath and magentically stirred while 5.5 g (0.036 mol) of α-terpineol (1) in 100 ml of methylene chloride was added over a two-hour period. The solution was then allowed to warm to room temperature, and 100 ml of a water solution of 2.8 g (0.056 eq) of sodium carbonate and 7.0 g (0.056 mol) of sodium sulfite was added dropwise over 10 min. The water solution, after it was stirred for 15 min, was weakly basic and gave a negative test with starch iodide paper.[14] The organic layer was separated, and the aqueous solution was extracted with two 100-ml volumes of ether. The combined organic extracts were dried over magnesium sulfate and filtered, and the solvent was removed at reduced pressure. A colorless oil, 5.6 g, was recovered. The two major components in this mixture were separated by vapor phase chromatography (10' x 3/8", 20% DEGS, 160°). The product with the shorter retention time (15 min) was a crystalline material that had the same physical and spectral properties and microanalysis as the recently reported 2-endo-cineolylol (9). Oxidation to the corresponding ketone via a chromium trioxide pyridine complex in methylene chloride[15] and formation of the corresponding oxime gave a mp 138°, reported 139°.[16] The product with the longer retention time (55 min) was an oil corresponding to p-menthane-α-1,2-epoxy-8-ol (7), bp 60° (0.2 mm); nmr (CCl_4), δ 1.08 (s, 6, CH_3), δ 1.25 (s, 3, CH_3), δ 2.98 (d, 1, H_2, $J_{2,3a}$ = 4.5 Hz), mass and ir spectra confirmatory.

Analysis -- Calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.60

Found: C, 70.63; H, 10.43.

p-Menthane-8-1,2-epoxy-8-ol (8) --
An analytical sample of the β-epoxide 8 is most readily prepared by the treatment of chlorohydrin 4 with base. In a 30-ml flask was placed 0.50 g (0.0025 mol) of chlorohydrin 4 and 25 ml of water. This mixture was cooled in an ice-water bath and magnetically stirred while 0.84 g (0.015 mol) of potassium hydroxide was added in small batches over a six-hour period. The mixture was then allowed to warm to room temperature, and the stirring was continued for another three hours. The aqueous mixture was extracted with four 25-ml volumes of ether. The ether extracts were dried over magnesium sulfate and filtered, and the ether was removed under reduced pressure. A short-path distillation produced 0.35 g of an oil, bp 55-60° (0.2 mm), nmr ($DCCl_3$), δ 1.15 (s, 6, CH_3), δ 1.13 (s, 3, CH_3), δ 3.06 (s, 1, H_8). mass and ir spectra confirmatory.

Analysis -- Calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66.

Found: C, 70.48; H, 10.57.

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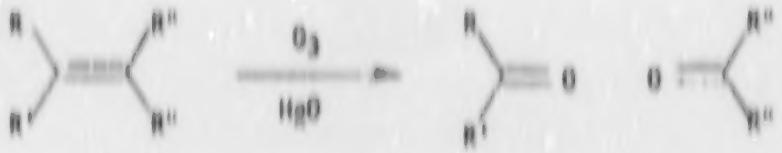
AQUEOUS OZONATION OF α-TERPINEOL

Results and Discussion

Ozonation appears to be a viable alternative to chlorination as a means of disinfection (Section I - Introduction). The effectiveness of ozone in water purification processes is in some respects, e.g., color and taste removal, disinfection, and reduction of BOD[1,2] superior to chlorine. However, in addition to the economic question, "Changing the disinfectant without an intense research input to study other public health ramifications could be a catastrophic step."[3]

Just as the olefinic center in α-terpineol is very susceptible to electrophilic attack upon aqueous chlorination, the olefinic center also reacts readily with electrophilic ozone. This interaction usually leads to the formation of products resulting from the cleavage of the carbon-carbon double bond.



Acids, etc. depending on nature substituent group

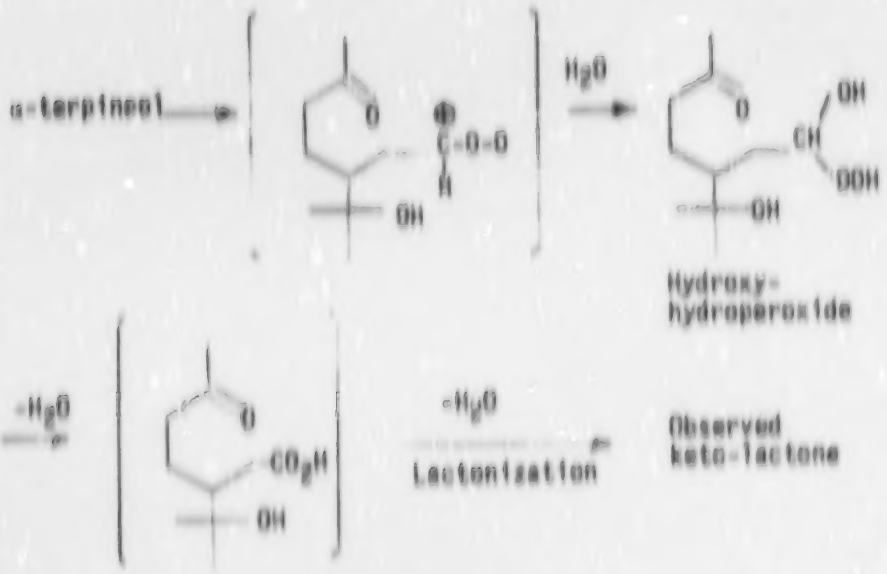
Possible non-cleavage products include epoxides, glycols, primary ozonides, normal ozonides, and tetra oxolanes.

It seems reasonable to compare the aqueous chlorination chemistry of α-terpineol with the aqueous ozonation chemistry. The major product upon the interaction of ozone in water with α-terpineol at either an acidic pH of 3 or basic pH of 10 is a keto-lactone.

α-terpineol

Keto-lactone derived from initial alkene cleavage

The formation of the keto-lactone is consistent with the Criegee Mechanism (Section I) via intermediacy of the hydroxy hydroperoxide.



An alternate mechanism for the formation of the keto-lactone via the intermediate keto aldehyde, an expected cleavage product, cannot be ruled out. The ketoaldehyde was synthesized via previously reported reductive conditions in dimethyl sulfoxide[4] and could be shown to be readily converted to the keto-lactone under aqueous ozonation conditions.

Other minor oxygenated cleavage products can also be detected in the aqueous ozonation of α-terpineol, but these products have yet to be unambiguously identified. It would be desirable to learn these structures to enhance our understanding of aqueous ozone chemistry and increase our predictive capabilities with other olefinic systems.[5]

Experimental

α-Terpineol was purchased from Matheson, Coleman and Bell Manufacturing Company and was fractionally distilled prior to use.

Procedure for the Ozonation of α-Terpineol --

Ten grams of α-terpineol (via magnetic stirring) with three liters of distilled water in a five-liter Erlenmeyer flask. An oxygen-ozone mixture was bubbled through the aqueous mixture with a sintered glass bubbler at a flow rate of 30 ml/min (corresponding to about 1.5×10^{-4} moles/min of ozone). The bubbling was continued for 7½ hours at 25°C. At the end of this time a KI-starch paper test indicated the presence of peroxidic material. The reaction mixture was allowed to stand overnight and then was slowly added to a 25-X 5-cm Amerlite XAD-2 column. The column was eluted with 50:50 ether/methanol, and 7.2 g of a crude product was obtained. The nmr and GC (OV-1, 5%, 120°) indicated the major product (75%) to be the keto-lactone α,α-dimethyl butyrolactone. The keto-lactone was purified by recrystallizing from hexane/ether, mp 60° (lit. 61°)[5]; ir, 1755, 1710, 1375, and 1390 cm^{-1}; ms, M^+ 184, 151, 123, 111, 98; nmr δ 1.28 s(3); δ 1.45 s (3), δ 2.2-2.6 m(3).

Analysis -- Calcd: C, 65.44; H, 8.86

Found: C, 65.19, H, 8.75.

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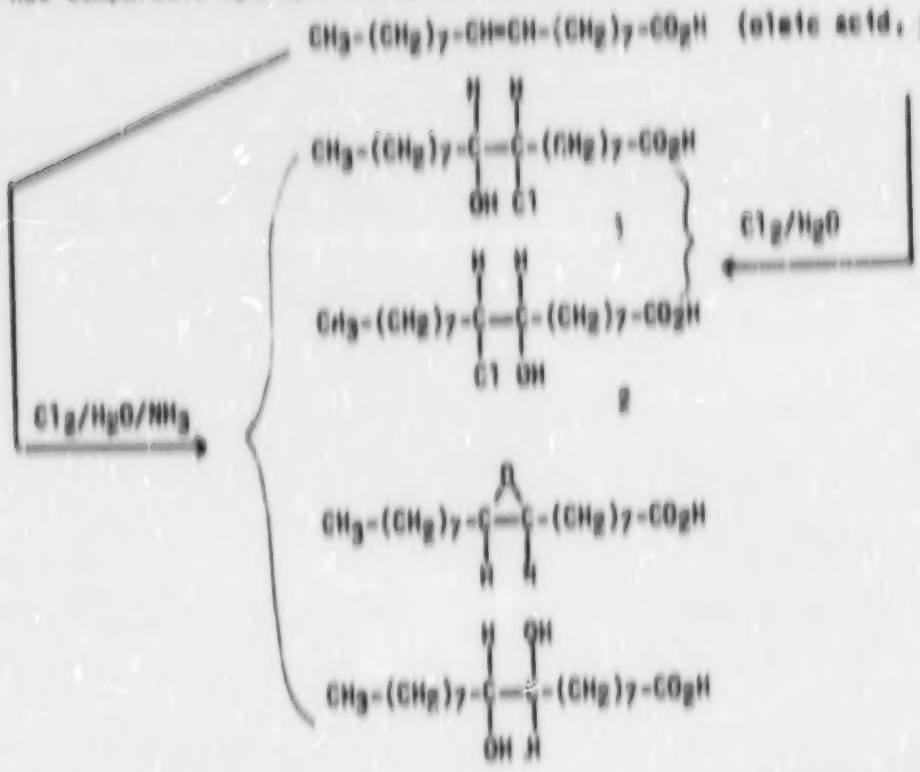
OLEIC ACID - AQUEOUS CHLORINATION

Results and Discussion

The reaction of oleic acid with chlorine[1] in distilled water produces a mixture of 9-chloro-10-hydroxystearic acid (1) and 10-chloro-9-hydroxystearic acid (2) over a wide range of pH values and chlorine concentrations (Table 6).

The presence of ammonia during the chlorination not only results in the formation of the chlorohydrin mixture but the generation of 9,10-epoxystearic acid[3], 9,10-dihydroxystearic acid[4], and an unidentified material that has comparable hydrophilicity to that of the glycol.



The chlorination of oleic acid produces a mixture of the 9,10-10,9-chlorohydrins under a variety of conditions. However, the change in product distribution due to the presence of ammonia is difficult to explain, as highly basic conditions do not produce the same results. In addition, dichlorostearic acid is absent even at low pH values.

A mixture of the two chlorohydrins is significantly toxic to Daphnia magna. The LC_{50} (48 hours) is 2-4 ppm, but the toxicity cannot be currently assigned to one or both of the isomers.

Experimental

All analyses were performed on a Waters Associates ALC 201 with 2 x 2' bondpak C-18 columns of Porasil B and a differential refractive index detector. The oleic acid was obtained from Nu-check-Prep Inc., Elysian, MN, in 99.9% purity.

Procedure --

Distilled water (or water containing NH_4OH) was saturated with oleic

TABLE 6. CHLORINATION PRODUCTS FROM OLEIC ACID

NaOCl (ppm)	Initial Oleic Acid (ppb)	Mixed Chlorohydrin (ppb)	Reaction (%)	pH
5.1	30	10	30±2	11.2
2.5	30	1	5±5	11.2
10.2	25	2	25±2	11.2
10.2	70	75	90±2	6.1
5.1	80	75	80±2	6.1
2.5	30	10	30±2	6.1
1.0	11	1	10±10	6.1
.51	10	NR	0	6.1
5.1	6	6	100	1.8
2.5	12	10	70±2	1.8
1.0	10	8	80±2	1.8
10.2	15	15	100	1.8

acid and adjusted to the required pH. Sodium hypochlorite was added and allowed to react for 15 min. After the addition of excess sodium sulfite to stop the reaction, the reaction was acidified and extracted with dichloromethane (3 x 60 ml). The resulting solution was dried over sodium sulfate, filtered, and evaporated *in vacuo*.

Threo-9,10-Dichlorostearic Acid --
The procedure of Pihlaja and Ketola[2] was followed: oleic acid (1.12 g) was added to 15 ml of CCl_4 at room temperature. To this mixture was added 40 ml of Cl_2 saturated in CCl_4 over a period of 15 min. After an additional 5 minutes the Cl_2 and CCl_4 were removed on a rotary evaporator. Petroleum ether (25 ml, 40-60°C) was added and the solution set aside to crystallize at -20°C. The crystals were collected by cold filtration [yield 0.56 g (40%) m; 36-37°C, literature[2] mp 37.4-37.8].

Cis-9,10-Epoxystearic Acid --
To a solution of oleic acid (1.1g) in dichloromethane (20 ml) was added a solution of 1.10 g of m-chloroperbenzoic acid in 12 ml of dichloromethane. The reaction mixture was stirred at room temperature for 2.5 hours and was then washed once with approximately 100 ml of a 10% sodium sulfite solution. The aqueous layer was discarded, the organic layer was dried over anhydrous sodium sulfate and filtered, and the dichloromethane was removed

on a rotary evaporator. The solid residue was crystallized from 25 ml of acetone (yield .85-.87 g (69-70%) mp 56-57.5°C, literature mp 59-59.8°C.[3])

Threo-10-Chloro-9-Hydroxystearic Acid and Threo-10-Hydroxy-9-Chlorostearic Acid Mixture --

The procedure was essentially that of McGhee et al.[4], in which concentrated hydrochloric acid (0.9 ml) was added to 9,10-epoxystearic acid (250 mg) in 10 ml of ether. After stirring for 45 minutes, the aqueous layer was decanted and the ether layer was washed with water to remove the mineral acid. The ether was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. After crystallization of the residue from 17 ml of petroleum ether (30-60°C) at -20°C, the crystals were collected (yield 191 mg (67%) mp 34-37°C, literature 33-41°C)[4].

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AQUEOUS OZONATION OF FATTY ACIDS (OLEIC AND LINOLEIC ACIDS)

Results and Discussion

The interaction of ozone with oleic acid was investigated to compare the aqueous ozone chemistry with the aqueous chlorine chemistry. As with α-terpineol, the reactive center in both of these reactions is the unsaturated olefinic linkage.

The major products in a typical aqueous ozonation of oleic acid are cleavage products of the olefinic linkage (Table 7). These products were followed by a GC analysis after methylation with diazomethane. The initial pH was about 3.9 and decreased slightly as the ozonation proceeded. The major cleavage products are aldehydic, but the ratio of products changes because of the subsequent autoxidation of the aldehydes with time. These cleavage products are those anticipated from the Criegee ozonolysis mechanism (Section I).

A non-cleavage ozonation product was also observed in the aqueous ozonation of oleic acid. Significantly, this product was identified as the epoxide of oleic acid (i.e., oleic acid oxide). The formation of epoxides from olefins is always of interest because of the potential carcinogenic properties of this functionality.[1-7] Preliminary studies on the origin of oleic acid oxide in this reaction indicate that it is not formed by direct epoxidation with ozone of oleic acid. It appears more likely that epoxide formation results from a subsequent peracid epoxidation of oleic

TABLE 7. AQUEOUS OZONATION PRODUCTS OF OLEIC ACID

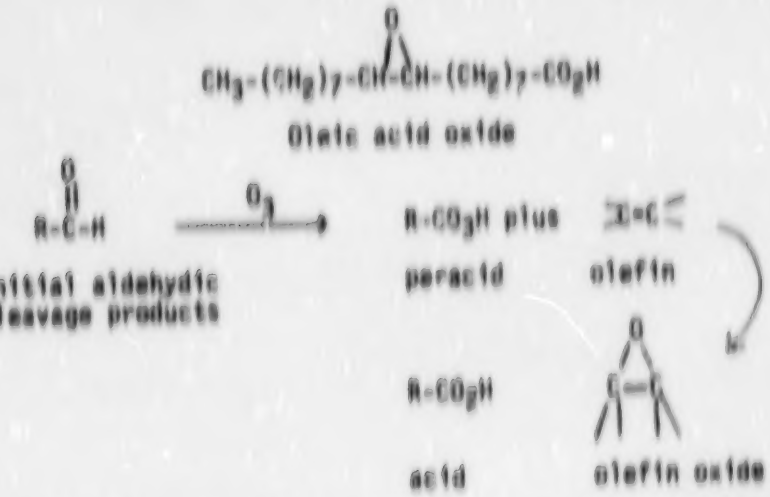
$$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H \xrightarrow[H_2O]{O_3} \xrightarrow[\text{methylation}]{CH_2N_2}$$

Oleic Acid (cis)

CH_3-$(CH_2)_7$-CH=CH$(CH_2)_7CO_2CH_3$	methyl oleate
$CH_3(CH_2)_7CHO$	n-nonyl aldehyde
$CH_3(CH_2)_6CO_2CH_3$	methyl caprylate
$CH_3O_2C(CH_2)_6CO_2CH_3$	dimethyl suberate
$CH_3(CH_2)_7$-CH(OH)-CH(OH)$(CH_2)_7$-CO_2CH_3	methyl 9,10-dihydroxystearate
$CH_3(CH_2)_7C(=O)$-CH_3	n-nonyl methyl ketone (via methyl insertion in n-nonyl-aldehyde)
$OHC(CH_2)_7CO_2CH_3$	methyl 9-oxononanoate
$CH_3C(=O)(CH_2)_7CO_2CH_3$	methyl ketone of above via methyl insertion
$CH_3O_2C(CH_2)_7CO_2CH_3$	dimethyl azelate

acid with the peracids arising from autoxidation of the aldehydic products.

The generality of this mechanistic possibility should be examined further.



The products identified in a similar aqueous ozonation study of linoleic acid are listed in Table 8. A number of minor products have yet to be identified in this reaction, a reaction that is more complex than oleic acid owing to the presence of two carbon-carbon double bonds. The cleavage products identified can be rationalized in terms of the Criegee ozonolysis mechanism. It will be desirable, however, to identify as many of the minor components as possible and again look for epoxide formation.

Experimental

Procedure --

The oleic acid was obtained from Nu-Check-Prep, Inc., Elysian, MN in 99.9% purity.

Oleic acid (3.70 g) was added to a two-liter Erlenmeyer flask containing 1,950 ml of distilled water. The pH of the saturated oleic acid solution was 3.9. The solution was stirred at room temperature, and an O_2-O_3 mixture was bubbled through the solution with a sintered glass bubbler. The O_2-O_3 flow rate was 30 ml/min, which corresponded to an O_3 flow rate of 30 ml/min. After 20 minutes, a 200-ml sample of the solution was removed and put into a 250-ml Erlenmeyer flask and stirred overnight to allow the nature, decomposition to products. Distilled water (200 ml) was added to the reaction flask to replace the volume lost. The ozonation was discontinued for approximately one minute while the first sample was being removed. After 40 minutes a second sample (220 ml) was taken; the same procedure was used as for the removal of the first sample. After 2 hrs 10 min, a third sample (380 ml) was taken, and after 3 hrs 35 min from the start of the ozonation, the ozonizer was turned off. After stirring overnight at

TABLE 8. AQUEOUS OZONATION PRODUCTS OF LINOLEIC ACID (LINOLIC ACID)

$CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_7CO_2H \xrightarrow[H_2O]{O_3} \xrightarrow[\text{methylation}]{CH_2N_2}$

$CH_3(CH_2)_4{-}CHO$	n-hexanal
$CH_3(CH_2)_4{-}\overset{O}{\overset{\|}{C}}{-}CH_3$	2-heptanone (via methyl insertion in n-hexanal)
$CH_3(-CH_2-)_4{-}CO_2CH_3$	methyl hexanoate
$OHC(-CH_2)_7CO_2CH_3$	methyl 9-oxopelargonate
$CH_3C(-CH_2)_7CO_2CH_3$	(via methyl insertion in above)
$CH_3OC(CH_2)_7CO_2CH_3$	dimethyl azelate
$CH_3(-CH_2-)_4{-}CH{=}CH{-}CH_2CHO$	3-nonenal

room temperature to allow for decomposition of peroxide intermediates, the samples were further acidified (1 ml of concentrated HCl for samples 1-3 and 3 ml of concentrated HCl for sample 4) and extracted with methylene chloride (samples 1-3 with three 40-ml portions and sample 4 with four 200-ml portions of CH_2Cl_2). The four methylene chloride solutions were then dried (anhydrous magnesium sulfate), filtered, and evaporated in vacuo. The product recovery was as follows: in sample 1, 0.39 g; in sample 2, 0.20 g; in sample 3, 0.43 g; in sample 4, 2.23 g, resulting in a total product recovery of 3.25 g.

In preparation for GC analysis the samples were exhaustively methylated with diazomethane. The ether-diazomethane solution was added to the samples until the green-yellow color of diazomethane persisted.

The GC analysis was accomplished by spiking the samples with a set of probable standard compounds. This was done on three different columns of different polarity. The columns and GC conditions were: 3% carbowax 20 M programmed from 80° to 230°C at 9°/min, a 3% phenyl silicone OV-25 programmed from 80° to 230° at 9°/min, and a silinated 5% methyl silicone OV-1 column programmed from 100° to 340° at 9°/min.

Of the compounds used as standards, methyl oleate was purchased from Nu-Check-Prep, Inc., octanoic acid, n-nonyl aldehyde, n-nonylic aldehyde, azelaic acid, suberic acid, and 9,10-dihydroxystearic acid were all purchased from IBC Pharmaceuticals, Inc., Plainview, NY. The acids were all methylated with diazomethane before use as standards on the GC. The other standard compounds, methyl azelaldehydate, and the methyl ketones of n-nonyl aldehyde and methyl azelaldehydate were synthesized by the procedure indicated below.

Methyl azelaldehydate -- This compound was made by the same procedure as used by G. Bozzato, J. Backmann and H. Pesari in making the hemiacetal from α-terpineol.[3] Methyl oleate, 24.55 g (6.69×10^{-2} moles) was put in a three-necked roundbottom flask containing 950 ml of absolute methanol. The solution was cooled to -70°C. An O_2-O_3 mixture was introduced into the reaction flask through a sintered glass bubbler at a flow rate of 30 ml/min, which corresponded to an O_3 flow rate of 4.2×10^{-4} moles/min. The ozonation was continued until the first trace of blue color (excess O_3) appeared. The solution was flushed with N_2 for 15 minutes, then 25 ml of dimethyl sulfide was added. The solution temperature was allowed to rise slowly to room temperature while flushing with N_2. After twelve hours of flushing with N_2 the methanol was removed in vacuo yielding 32.6 g of product. After washing with sodium bicarbonate, samples of n-nonyl aldehyde and methyl azelaldehydate were obtained in >95% purity (by GC) by vacuum distillation. Nonanal was distilled over at 30°C (1.0 mm) and methyl azelaldehydate at 78-82°C (0.9 mm). The structures were confirmed by nmr and ir.

Methyl ketones of nonanal and methyl azelaldehydate -- Diazomethane was added to each of two 250-ml Erlenmeyer flasks. One flask contained 1 g of nonanal and the other contained 1 g of methyl azelaldehydate. The solutions were stoppered loosely and allowed to stand overnight (15 hours), after which the products were concentrated in vacuo. Nmr analysis showed the product to be the methyl ketones of the corresponding aldehydes. The purity of the nonylmethyl ketone was >90% and that of the methyl ketone of methyl azelaldehydate was >85% (by GC).

Methyl oleate oxide -- Oleic acid (1.16 g) in 20 ml of dichloromethane was added to a solution of m-chloperbenzoic acid (1.10 g) in 12 ml of dichloromethane. The mixture was stirred at room temperature for 2.5 hrs and then washed once with 100 ml of 10% sodium sulfite solution. The aqueous layer was discarded and the organic layer dried. The solvent was removed in vacuo leaving a solid residue that was crystallized from 25 ml of acetone, mp 56-57.5°C (lit. mp 59-59.5°C). The cis-9,10-epoxystearic acid was then methylated with diazomethane to methyl oleate epoxide.

The linoleic acid was obtained from Nu-Check-Prep, Inc., Elysian, MN in 99.9% purity.

Procedure --

Linoleic acid (1.20 g) was added to a two-liter Erlenmeyer flask containing 1,000 ml of distilled water. The initial pH of the saturated linoleic acid solution was 4.3. The ozonation was carried out at room temperature and with a O_2-O_3 flow rate of 30 ml/min corresponding to an ozone flow rate of 7.8×10^{-5} moles O_3/min. The pH at the end of the ozonation was 3.8. The sampling and extraction procedure was the same as that described for oleic acid, the only difference being that the peroxide intermediates were given three days to decompose instead of one. A test with KI starch paper indicated that decomposition was not complete after one day. The sampling and extraction procedures used on the three samples taken were as follows:

Sample	Time ozonated	Amount H_2O sol removed	Amount CH_2Cl_2	Extracted with	Amount product removed
1	20 min	350	200	4 fractions	0.25 g
2	50 min	350	200	4 fractions	0.25 g
3	165 min	1,950	800	4 fractions	0.68 g
				Total product recovery	1.18 g

The samples were then methylated with diazomethane in preparation for GC analysis.

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CHLORINATION OF RESIN ACIDS

Results and Discussion

Diterpene "resin acids" ($C_{19}H_{29}CO_2H$) represent an additional compound type often present in waters subjected to aqueous chlorination (e.g., the bleaching of wood products or the disinfection of certain natural or industrial waters).

The various chlorination products referred to in the following discussion of the chlorination of dehydroabietic and abietic acids are graphically presented in Figure 2. Numbered compounds in the text can be identified by reference to the figure.

Chlorination of dehydroabietic acid (Compound 1) with a large excess of chlorine gave products that could not be separated by crystallization or silica gel chromatography. The reaction mixture was, therefore, converted to the methyl esters with diazomethane, which in turn led to the isolation of the esterified Compounds 3 and 4 by fractional crystallization. Low concentration chlorination (6 equivalents) of dehydroabietic acid gave products identical to those from high concentration chlorination as shown by high pressure liquid chromatography (HPLC). The chlorine uptake (i.e., yield of products) was quite high (38% product formation).

Chlorination of abietic acid (pH 2) (Compound 8) and subsequent column chromatography gave rise to one major and two minor product-containing fractions. The major fraction was esterified with diazomethane and then separated into two components by preparative layer chromatography (PLC). One of these was assigned structure 5, and the other was shown to be identical to Compound 4.

Compound 5 exhibited a characteristic AB pattern for the olefinic protons, but these were absent in Compound 4. On the other hand, Compound 4 possessed a one-proton quartet expected for the rigid ring proton of the type Ar-CH-Cl-CH_2-. The two aromatic protons of Compound 3 occurred as a two-proton singlet in $CDCl_3$, and in acetone two one-proton singlets were observed. Thus, the chlorine must be placed on the ring so that an AB pattern cannot be observed in the nmr spectrum.

Structures 3, 4 and 5 were also established by interconversion. Compound 5, when treated with HCl gas, gave Compound 4. Treatment of Compound 4 with Cl_2 gas gave Compound 3.

One of the polar compounds formed in the high concentration and low pH chlorination was not observed (HPLC) when the reaction was carried out in dilute solution with one equivalent of chlorine. The empirical formula suggests a mono epoxy chlorohydrin of the type 7. When the chlorination was carried out as described in the literature for dichlorodihydroxyabietic acid (Compound 6) the major product was identical to the one formed in the chlorination carried out under mild conditions at pH 2.

The acids 8 and 10 corresponding to esters 5 and 3 respectively were also prepared by independent synthesis. Mercuric acetate oxidation of abietic acid followed by basic hydrolysis gave the olefin 8. Ferric-chloride -catalyzed chlorination of Compound 9 gave dichlorodehydroabietic acid (Compound 10) in high yield. The chlorination product, Compound 9, of dehydroabietic acid in the absence of $FeCl_3$ was the monochlorinated isomer, which could be purified on a silica column.

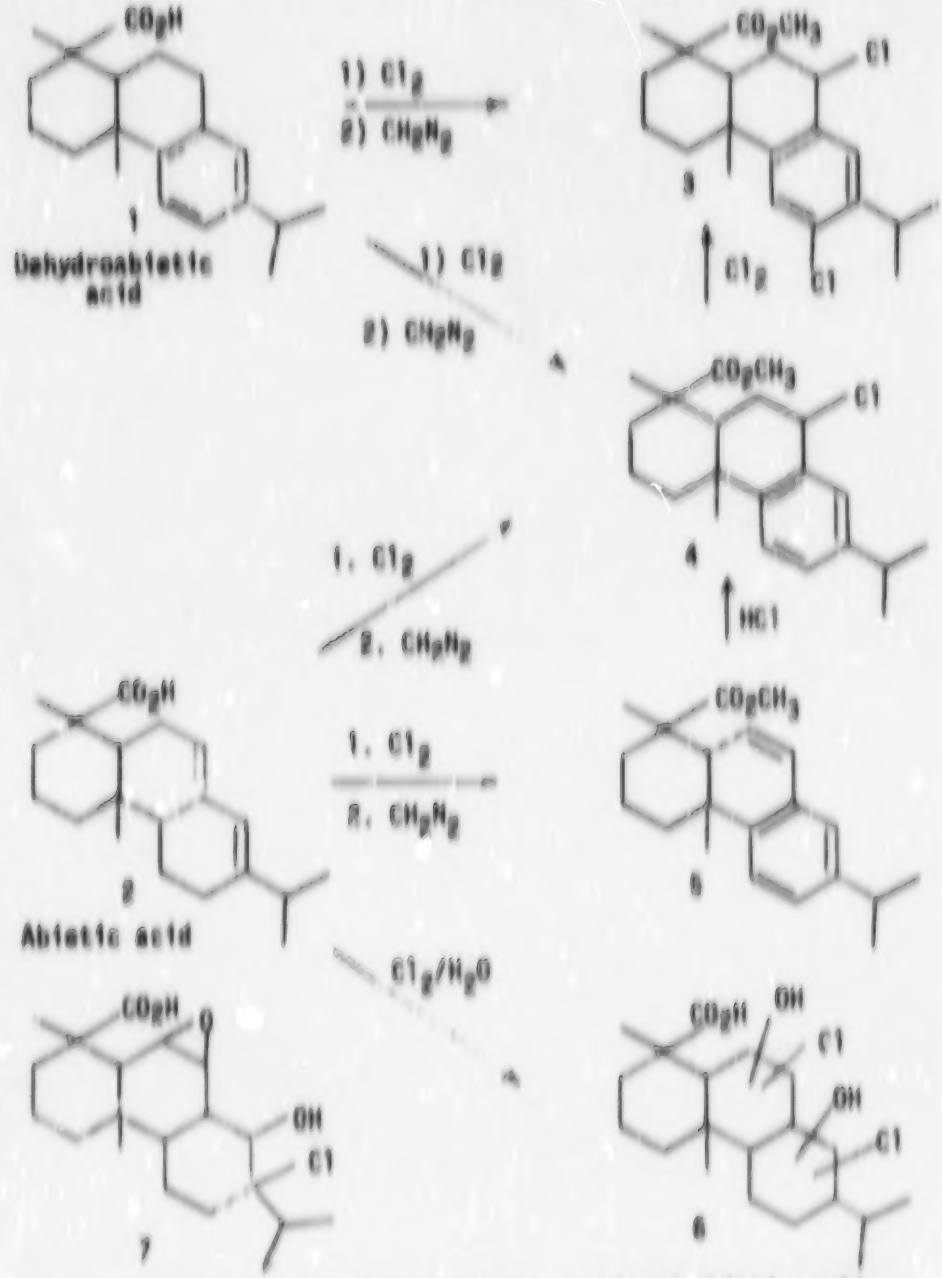

Figure 2. The chlorination of dehydroabietic acid and abietic acid

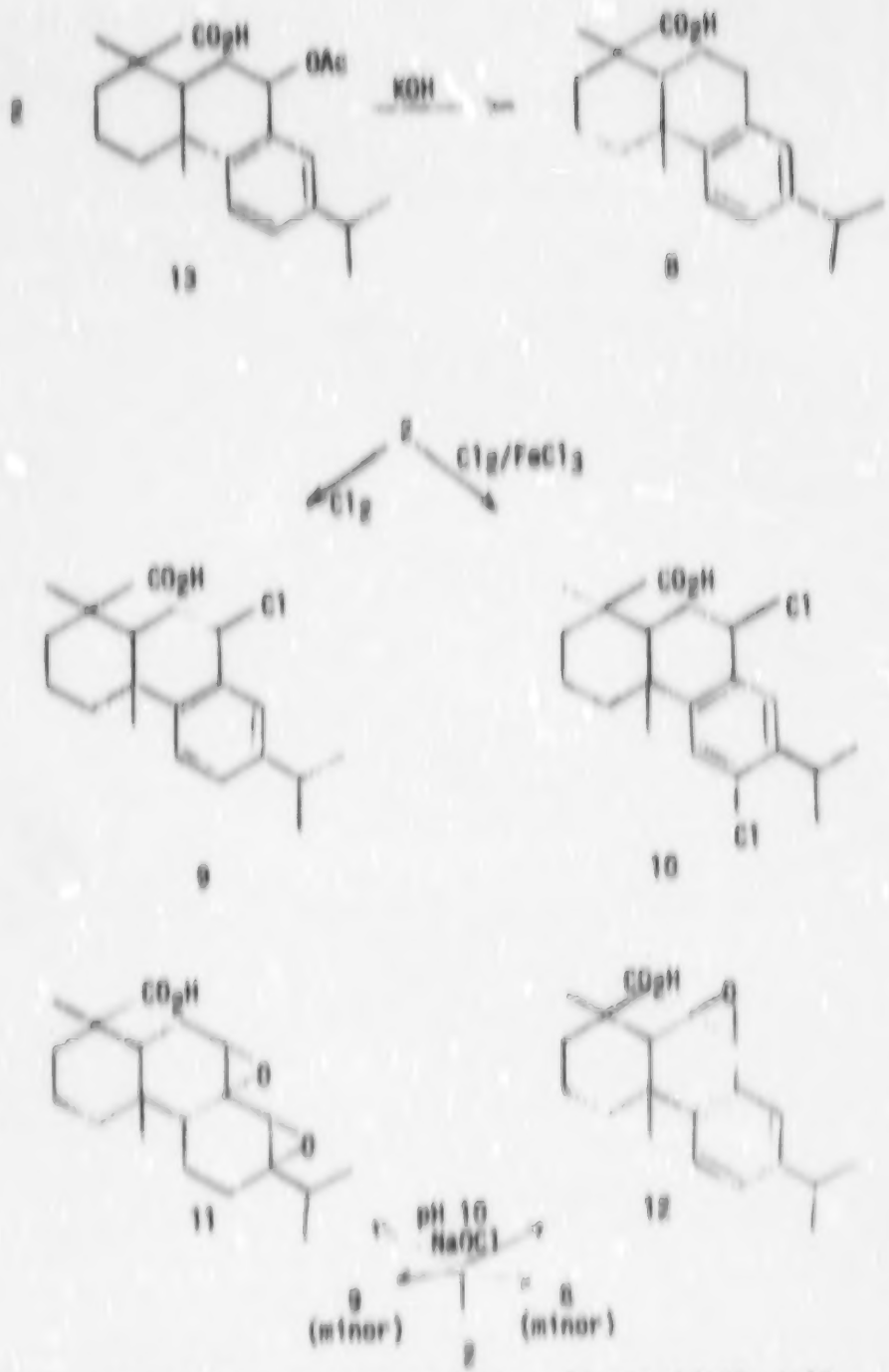

Figure 2. The chlorination of dehydroabietic acid and abietic acid - continued.

When the chlorination of abietic acid was carried out at pH 10, Compounds 6 and 9 were formed as minor products, with the major products being the diepoxide (Compound 11) and an aromatized compound tentatively assigned structure 12. Table 9 outlines the overall product distribution with pH.

TABLE 9. PERCENTAGE OF COMPOUNDS FORMED BY CHLORINATION OF ABIETIC ACID AT VARIOUS pH VALUES[a,b]

Compound	pH 2	4	6	8	10	Retention time (min)
Diepoxide (11)	0	0	4	30	32	8.8
Olefinic (5)	66	78	71	4	4	10.1
Monepoxide (12)	0	3	13	52	50	13.2
Monochloride (9)	12	9	6	12	19	15.2
Polar component (7)	22	10	6	0	0	20.6

[a] Reaction conditions: 1 g, water containing 10 mg of abietic acid treated with two equivalents Cl_2 as NaOCl for 0.5 hr.

[b] HPLC conditions: Solvent, 4% 1-propanol, 44% hexane, 52% ether; column, 3 porasil columns; flow rate, 1 ml/min.

Experimental

Dehydroabietic Acid Purification --

Technical dehydroabietic acid (5 g) was dissolved in acetone (10 g), and 2-amino-2-methyl-1-propanol was added. Crystals appeared immediately, and the mixture was allowed to stand for 24 hours. The salt was recrystallized from ethanol (4.1 g, mp 180-191°). Saturated boric acid (5 ml) was then added to a suspension of the salt in ether. After stirring for one hour, the ether layer was separated and washed with water. Evaporation of the ether gave dehydroabietic acid that was crystallized from ethanol water (4/1) (3 g, mp 172°C, lit. 171-173°[2]); nmr ($CDCl_3$) δ 1.23 (s, 3, CH_3), δ 1.17, (s, 3, CH_3).

Abietic Acid Purification --

Technical abietic acid (24.5 g) was dissolved in acetone (37.5 ml), and diamylamine (12.7 g) was added slowly to the hot solution. Upon cooling to room temperature, crystals formed and the mixture was cooled overnight. The solvents were removed by filtration, and after four recrystallizations from acetone the salt melted sharply at 134°. This salt (5.4 g) was dissolved in 40 ml of ethanol and cooled in the refrigerator. Acetic acid (1.4 ml) and water (20 ml) were added, and the mixture was allowed to remain cold for an additional 24 hours. The resulting abietic acid amounted to 3.6 g

[mp 179-172°, lit. 171-173°[9], nmr ($CDCl_3$) δ 1.04 (s, 3, CH_3), δ .94 (s, 3, CH_3) δ .88 (s, 3, CH_3).]

High Concentration Chlorination of Dehydroabietic Acid --
Dehydroabietic acid (0.5 g) was dissolved in ether (10 ml) and mixed with water (500 ml) while chlorine gas was passed through the solution for one-half hour at a flow rate of 20 ml/min. After standing for twelve hours, the mixture was extracted with two 100-ml portions of ether. Evaporation of the ether left an oily residue (0.6 g). Attempts to crystallize or separate the products by thin layer chromatography failed.

The reaction mixture was then methylated with excess diazomethane in ether. Evaporation of the solvent left a solid glass, which was dissolved in a minimum amount of hot ethanol. After standing in the refrigerator overnight, the crystalline product was collected by filtration. Compound 3, 11 mg, mp 138-140°C; nmr ($CDCl_3$) δ 7.12 (s, 2, aromatic), δ 3.67 (s, $COOCH_3$), δ 3.38 (q, 1, $ArCHCl$), δ 1.29 (s, 6, 2 CH_3), δ 1.18 (s, 3, CH_3), δ 1.16 (s, 3, CH_3); nmr (acetone D_6) δ 7.19 (s, 1, aromatic), δ 7.34 (s, 1, aromatic). The filtrate obtained from the isolation of Compound 3 was evaporated to dryness, and the residue was dissolved in a minimum amount of hot ether. A crystalline solid formed after the ether evaporated at room temperature. Recrystallization gave Compound 4, 120 mg, mp 110-111°, nmr ($CDCl_3$) δ 7.00 (s, 3, aromatic), δ 3.67 (s, 3, $COOCH_3$), δ 3.38 (q, 1, $ArCHCl$), δ 1.27 (s, 6, 2 CH_3), δ 1.20 (s, 3, CH_3) δ 1.16 (s, 3, CH_3). Compounds 3 and 4 showed molecular ions at M/e 382 and 348 respectively.

High Concentration Chlorination of Abietic Acid --
The chlorination of abietic acid was carried out as described for dehydroabietic acid. Thin layer (benzene/acetone/acetic acid; 40 cc/1cc/8 drops) showed three spots when developed twice. The Rf values were 0.71, 0.57 and 0.43. The crude reaction mixture (1.7 g) was applied to a silica column (90 g) and was eluted with benzene/HOAc (600/1). After the first 50 ml were discarded, 160, 13-ml, fractions were collected at a flow rate of 16 drops/min. Fractions were combined as follows: A, 1-67, 0.5 g; B, 68-89, 0.8 g; C, 90-120, 0.030 g; D, 121-158, 0.025 g. Fractions A, B, C and D correspond to unreacted abietic acid, and compounds with Rf 0.71, 0.57 and 0.43 respectively.

The major fraction B (0.2 g) was methylated with excess diazomethane in ether. The residue was subjected to preparative thick layer chromatography with triple development (hexane/benzene, 10/1). A compound of yet unknown structure (40 mg), Rf 0.15, and Compound 5 (140 mg), Rf 0.2, were isolated. Compound 5, nmr ($CDCl_3$) δ 7.00 (m, 3, aromatic), δ 6.09 (AB, 2, HC=CH, $\Delta\nu_{AB}$ 61 cps, J_{AB} 10 cps); molecular ion M/e 322.

Interconversion of Compounds 3 and 4 --
A mixture of Compound 4 (40 mg) in ether (1 ml) and water (100 ml) was treated with chlorine gas (20 ml/min) for 0.5 hr. The mixture was then extracted with two 50-ml portions of ether. Evaporation of the solvent left a glass which was dissolved in a minimum amount of hot ethanol and allowed to stand in the cold overnight. A crystalline product (16 mg) was collected

that was shown by mp and the mixed mp to be identical to Compound 3.

Interconversion of Compounds 4 and 5 --
Compound 5 (100 mg) was dissolved in methylene chloride (15 ml) and cooled at 0° while anhydrous HCl gas was bubbled through the mixture for 15 minutes. The mixture was stoppered and allowed to stand in the freezer overnight. The partially crystallized mixture was triturated with ether, and a crystalline product (6 mg) was collected that was shown by mp and mixed mp to be identical to Compound 4. The reaction was observed (nmr) to be 50% complete.

Low Concentration Chlorination of Dehydroabietic Acid --
A saturated solution of dehydroabietic acid (5.3 mg/l, 1.70×10^{-5}m) was treated with 7.3 mg of Cl_2 (6 equivalents), for 0.5 hr. Sodium thiosulfate was added, and the reaction mixture was extracted twice with ether. The residue was treated with diazomethane and subjected to liquid chromatography with cyclohexane/chloroform (25/1). The retention times of pure samples of the methyl esters of dehydroabietic acid, Compound 3, and Compound 4 were respectively 7.3, 8.3 and 11.6 minutes at a flow rate of 0.3 ml/min. Known 50/50 mixtures of the compounds showed nearly identical response to refractive index measurements. The reaction mixture contained unreacted starting material, Compound 4, and Compound 3 in amounts of 62%, 32% and 6% respectively.

Independent Synthesis of Compound 6 --
Red mercuric oxide (1.43 g) was heated in acetic acid (3.3 ml) and acetic anhydride (0.66 g) for 1.5 hours. At the end of this time, abietic acid (1 g) in acetic acid (3.3 ml) was added, and the mixture was refluxed for 1.5 hours. The mixture was cooled, and the metallic mercury was removed by filtration through a sintered glass funnel. The filtrate was mixed with water (50 ml) and extracted with two 20-ml portions of ether. The residue obtained by evaporation of the ether was dissolved in a minimum amount of hot methanol, allowed to cool to room temperature and then placed in the freezer overnight. The crystalline acetate, Compound 13 (0.32 g) was removed by filtration; mp 203-204°, lit.[3] 202-203°C.

Compound 13 was boiled in a 1 N solution of KOH in ethanol (20 ml) for two hours. At the end of this period, the mixture was cooled and made slighly acidic with concentrated HCl. The ethanol was evaporated, and the residue was dissolved in ether. The organic layer was washed with water, and evaporation of the solvent left a solid glass which was dissolved in hot ethanol. The ethanol was cooled to give a mp 170, lit.[3].

Isolation of Compound 9 --
The chlorinated reaction mixture of dehydroabietic acid showed no separation on tlc. However, a column chromatography was run (90 g silica gel) with benzene/acetone/acetic acid (400ml/10ml/40drops) at a flow rate of 20 drops/min. Analysis of the nmr spectra showed that fractions 25-42 contained only the monochloride, Compound 9. A sample of the monochloride that was with excess diazomethane had the same melting point as Compound 4.

Independent Synthesis of Compound 10 --
Compound 9 (0.1 g) was dissolved in carbontetrachloride (15 ml), and a catalytic amount of ferric chloride was added. Chlorine was bubbled through the solution for one minute and the mixture was stoppered and allowed to stand for three hours. The mixture was then extracted with two 10-ml portions of water, and the carbon tetrachloride was evaporated to give a product that was assigned structure 10 by its nmr spectrum.

Alternate Synthesis of the Polar Abietic Acid Chlorination Product (Rf 0.43) -- Literature Synthesis of Dichloro Dihydroxy Abietic Acid --
Abietic acid (0.3 g) was dissolved in $CHCl_3$ (5 ml) and acetic anhydride (0.33 ml). A solution of sodium acetate (2.5 g) in sodium hypochlorite (5%, 5.1 ml in 8 ml of water) was added, and the mixture was allowed to stir for one hour. The organic layer was then extracted with two 10-ml portions of water, and the $CHCl_3$ was evaporated. The residue (0.35 g) was subjected to column chromatography (45 g silica gel 60) with benzene/acetone/acetic acid (40/1/5 drops). The chromatography was run at a flow rate of 20 rops/min, and fractions were collected every 25 min. Fractions 35-46 contained 0.15 g of the compound (Rf 0.43) that had been isolated from the direct chlorination of abietic acid (pH 2). The literature[4] reported the major product of this reaction to be dichlorodihydroabietic acid.

Reaction of Abietic Acid with Sodium Hypochlorite at pH 10 --
Abietic acid (0.5 g) was dissolved in three liters of water containing sodium hypochlorite (9.8 ml of 5%, 4 mole, 2 eq.), and the pH was adjusted to 10 with sodium hydroxide. After the mixture stirred for one hour, the pH was lowered to 5.8 with dilute HCl, and the mixture was extracted with two 250-ml portions of ether. The ether was evaporated, and an oil (0.6 g) was obtained. The residue obtained from four such reactions (2.3 g) was subjected to column chromatography on silica gel 60 column (90 g). The flow rate was 10 drops/min, and seventy fractions were combined and contained respectively 0.23 g, 0.41 g, and 0.1 g. Fraction C was shown by nmr to contain olefinic compound 8 and a small amount of monochlorodehydroabietic acid Compound 9. Fraction A (Compound 11) was dissolved in a minimum of hot ethanol, and the crystalline material was removed by filtration; mp 150-153°; nmr ($CDCl_3$) δ 1.20 (s, 3, CH_3), δ 1.01 (s, 3, CH_3), δ 0.87 (s, 3, CH_3), δ 0.75 (s, 3, CH_3), δ 2.86 (m, 2, O-CH); mass spec.: methyl ester, M/346. Fraction B, i.e., proposed Compound 12, was dissolved in a minimum amount of hot ethanol, and the crystalline material was removed by filtration; mp 155-160°; nmr ($CDCl_3$) δ 1.26 (s, 3, CH_3), δ 1.20 (s, 3, CH_3), δ 1.28 (s, 6, CH_3), δ 2.89 (s, 2, O-CH).

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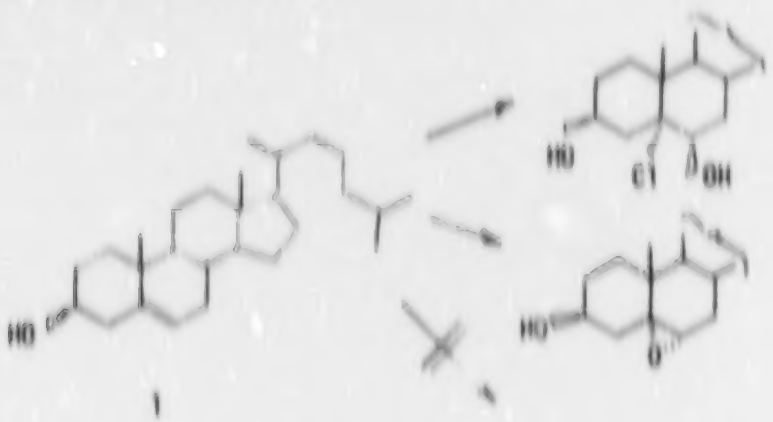
CHOLESTEROL

Results and Discussion

Chloresterol (I) was subjected to aqueous chlorination at a number of pH values and at both high and low concentrations of chlorine. The products that formed corresponded to known samples of the 5-α-chloro-3β, 6β-diol (II) (the anticipated chlorohydrin!) and the α-epoxide (III). Other possible products such as the dichlorides, the isomeric chlorohydrin, and the triol,[2] were not observed.



I

The chlorination products of cholesterol follow the expected stereochemical patterns characteristic of the rigid steroid system whereby the incoming reagent approaches from the less hindered (i.e., lower) α side. The formation of the epoxide in this situation (as with terpineol) may well be due to the presence of the six-membered ring in the presumed intermediate chlorohydrin which would maintain the molecule in a proper conformation for subsequent epoxide formation.

The formation of only two products in the present experiment differs considerably from the results reported by Lindgran[1]. Lindgran observed no epoxide, but other compounds (including ketonic oxidation products) were isolated. The difference in experimental conditions appears to be the most significant difference in the two studies. The reaction was performed in these laboratories in dilute aqueous media, whereas the earlier study was carried out at higher concentrations in aqueous butanol.

TABLE 10. PERCENTAGE OF COMPOUNDS FORMED BY CHLORINATION OF CHOLESTEROL AT VARIOUS pH VALUES

Summary of Results

pH	Cholesterol (1)	Chlorohydrin (2)	α-Epoxide (3)
2.2	20%	80%	
4.0	70%	-	30%
7.2	80%	20%	-
10.0	88%	10%	5%

Experimental

The cholesterol was recrystallized from methanol before chlorination.

Reaction Procedure --
A stock solution of cholesterol in water (10 liters) was made up by saturating the water with cholesterol and stirring for a week to assure saturation (2.6 ppm). The cholesterol solution was filtered to remove excess cholesterol, and the pH was adjusted by addition of HCl. The NaOCl was added at approximately two millimole of NaOCl to one millimole of cholesterol. The reaction mixture was stirred and allowed to run for 20 minutes. Sodium sulfite was used to stop the reaction (10 to 1 excess). The reaction mixture was then extracted with ether, dried over magnesium sulfate, and evaporated. The product distribution was analyzed by high-pressure liquid chromatography.

5α-chlorocholestan-3β, 6β-diol[2] --
To 8.0 g cholesterol in 100 ml of ether was added 35 g $Ca(OCl)_2$ in 600 ml of H_2O with stirring. Then 15 ml of acetic acid was cautiously added, and the mixture stirred for 30 minutes. The ether was washed with sodium sulfite solution and water and dried over magnesium sulfate. The ether was evaporated, and the product was recrystallized with MeOH (mp 145-147°C).

α-Epoxide[3] --
The α-epoxide was made by a modification of the published procedure. A cold solution of m-chloroperoxybenzoic acid (2.175 g) in ether (35 ml) was added to cholesterol (3.105 g) in 20 ml of dry ether and allowed to stand for 24 hours at -5°C. The solution was washed with ice water, sodium bicarbonate, water 5% ferrous sulfate solution, and water. Then ether was dried over magnesium sulfate, evaporated, and recrystallized from MeOH (mp 141.0-142.0°C).

Dichloride[4] --
Two grams of cholesterol was dissolved in 20 ml of $CHCl_3$ containing

0.08 g antimony trichloride and cooled to -20°C. In another flask 200 ml of $CHCl_3$ were saturated with chlorine by bubbling chlorine gas into the solution. The chlorine solution was added to the cholesterol solution until the yellow color remained. The chloroform solution was washed successively with sodium carbonate solution, 1 N HCl and water and dried over magnesium sulfate. The product was recrystallized from MeOH-ethyl acetate (mp 136.5-137.5°C).

6β-chlorocholestan-3α, 5α-diol[5] --

To one gram of cholesterol epoxide in 50 ml of tetrahydrofuran was added 1 ml of concentrated HCl with stirring. After 12 hours the solution was extracted with ether, washed with water, and dried over magnesium sulfate (mp 138.5-140.0° from methanol). The 6α-chlorocholestan-3α, 6α-diol isomer can be obtained from the recrystallization solvent in small quantities.

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PREPARATIVE METHODS FOR CHLOROPHENYL PHENOL SYNTHESIS

Results and Discussion

The synthesis of chlorophenyl phenols was initiated to provide additional examples of phenols suitable for inclusion in our study of chemical structure-biological activity relationships. Moreover, the availability of such a synthetic capability would have value for the preparation of possible polychlorinated biphenyl (PCB) metabolic products[1], which as examples of chlorophenyl phenols would be expected to exhibit herbicidal, fungicidal and antibacterial activity.[2-18]

The effort expended on the synthesis of these phenols was limited because of the insolubility of the polychlorinated products in water and the accompanying difficulty in pursuing valid toxicity measurements.

Several simple chloro analogs of 4-phenylphenol were synthesized by methods (or variations of methods) previously reported[1-4,16-19].

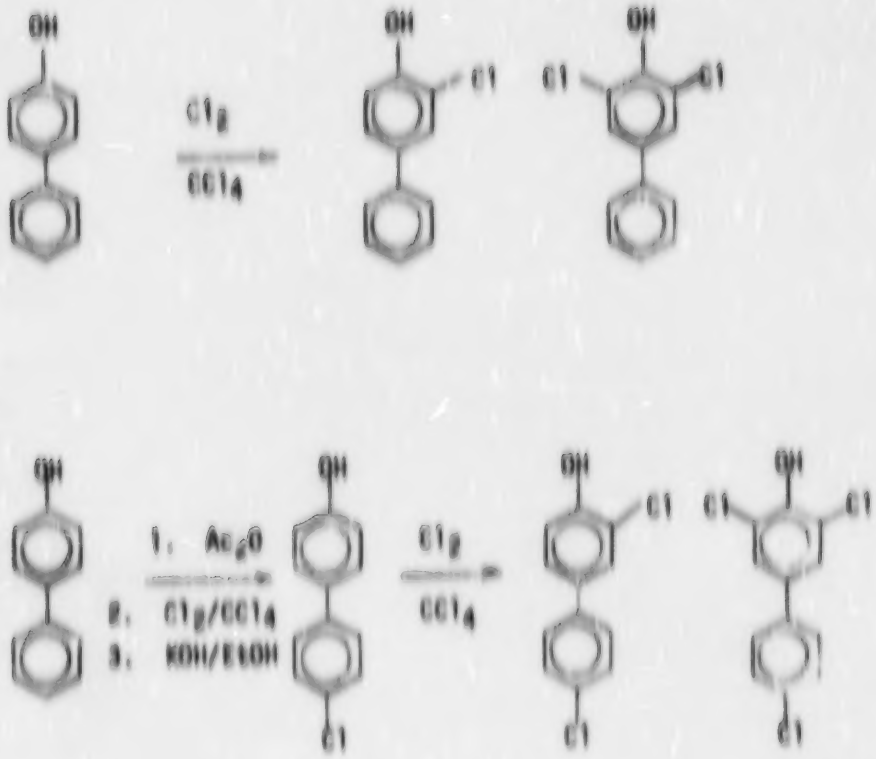
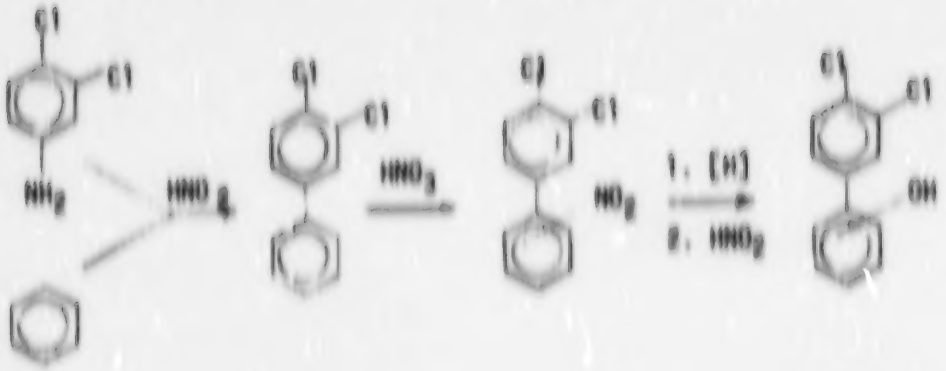


Figure 3. Chlorophenylphenol synthesis.

A direct translation of the above procedure to 2-phenylphenol was unsuccessful because the anticipated chlorination in the ring bearing the acetate did not occur. In addition, diazo coupling with a chlorophenol only produced the diazine. An alternative approach to direct chlorination was, therefore, used[20-24], in which the phenolic-OH was introduced in the last step through nitration, reduction, diazotization, and hydrolysis of a biphenyl already bearing the desired chlorination pattern. This approach took advantage of the ready availability of the required chloroanilines and the anticipated nitration of the biphenyl nucleus in the ring not bearing the chlorines.

Experimental

1(3',4'-dichlorophenyl)-3,3-dimethyltriazene --
To a one-liter beaker containing 32.4 g (0.2 mole) of recrystallized 3,4-dichloroaniline were added 25 ml of concentrated hydrochloric acid and 50 ml of water. This mixture was heated (85°C) to dissolve the 3,4-di-



chloroaniline. At the point where solution was achieved, another 55 ml of concentrated hydrochloric acid was added. The solution was immediately cooled to -3 to -5°C. A thermometer was placed in the beaker, and stirring was continued. A solution of 14.4 g (0.21 mole) of sodium nitrite was added dropwise and below the surface by means of a long-stemmed separatory funnel at a rate to maintain the temperature below 0°C. The stirring was continued for 15 minutes after the sodium nitrite addition was complete. A urea solution (1 g in 5 ml of water) was slowly added to decompose the excess nitrite, and stirring and cooling were continued.

A solution was prepared containing 250 ml of water and 87 g of sodium carbonate. To this was added enough crushed ice to lower the temperature to 10°C. 27.0 g of dimethylamine (Aldrich, 40% aqueous) was added, and then the diazonium salt solution was added to this solution dropwise and under the surface. The temperature was maintained at 10°C by the addition of more ice. After the addition was complete, stirring was continued for 15 minutes. The triazene precipitated and was recrystallized from ethanol (95%) to yield 32 g (73%).

1(2',4'-dichlorophenyl)-3,3-dimethyltriazene and 1(3',5'-dichlorophenyl)-3,3-dimethyltriazene --

These two compounds were prepared in the same manner as described above. 2,4-dichloroaniline resulted in an 89% yield. 3,5-dichloroaniline gave an 86% yield.

A mixture of 1.09 g (5 moles) of 1-(3',4'-dichlorophenyl)-3,3-dimethyltriazene and 3.2 g (20 moles) of 2,6-dichlorophenol was heated as liquids (5 hours). Thin-layer chromatography indicated one major product.

3,4-dichlorobiphenyl --

To a 250-ml round bottom flask was added 11.4 g (0.052 mole) of 1-(3',4'-dichlorophenyl)-3,3-dimethyltriazene, 16.2 g (0.07 mole) of anhydrous camphorsulfonic acid, and 100 ml of benzene. This mixture was reflux-

ed for two hours, allowed to cool, and poured into 200 ml of cold water. The benzene layer was separated, washed with saturated aqueous sodium bicarbonate, then washed with water, and then dried over sodium sulfate and concentrated (9.5 g).

The concentrated material was dissolved in petroleum ether (30-60°) and filtered through a silica-gel column (80 g) to yield 7.2 g (62%) of 3,4-dichlorobiphenyl.

2,4-dichlorobiphenyl and 3,5-dichlorobiphenyl --
These two compounds were prepared in the same manner as described above. The yields were 70% and 65% respectively.

2- and 4-(3',4'-dichlorophenyl)-nitrobenzene --
To a 100-ml round-bottom flask were added 12.2 g (0.051 mole) of 3,4-dichlorobiphenyl, 30 ml of glacial acetic acid and 15 ml of concentrated nitric acid. After refluxing for eight hours (130°-135°), the reaction mixture crystallized upon cooling. The crystals were washed well with water and then recrystallized from ethanol to yield 12.3 g (10%) of a mixture of the ortho- and para-substituted compounds. Thin-layer chromatography and nmr indicated the para- substituted product to be the major component.

2- and 4-(3',4'-dichlorophenyl) aniline --
Procedure A -- To a 25-ml round-bottom flask were added 500 mg (1.9 moles) of the ortho/para (3',4'-dichlorophenyl) nitrobenzene mixture, 5 ml of methanol, 2 ml of concentrated hydrochloric acid and 1 g of iron powder. This mixture was allowed to reflux for 12 hours, cooled, then extracted with benzene, washed with saturated sodium bicarbonate and then with water, dried over sodium sulfate and concentrated to yield 350 mg of oil. Thin-layer chromatography indicated no starting material and prep TLC yielded 150 mg (34%) of 4-(3',4'-dichlorophenyl) aniline.

Procedure B -- To a 250-ml Parr hydrogenation bottle were added 5.2 g (19 mmoles) of the ortho/para-(3',4'-dichlorophenyl)-nitrobenzene mixture, 50 ml of absolute ethanol and 300 mg of 10% Pd/C. The bottle was placed in a Parr hydrogenation apparatus and was shaken until hydrogen uptake was complete (15 min). The solution was filtered, and the ethanol was removed to yield 4.5 g of oil. Thin-layer chromatography indicated two major spots.

2-phenylphenyl acetate --
To a 250-ml round-bottom flask set up for reflux were added 42.5 g (0.25 mole) of 2-phenylphenol, 76 g (0.75 mole) of acetic anhydride, and 0.6 g of sodium acetate. This mixture was refluxed for three hours, cooled, poured into 750 ml of water, and allowed to stand overnight. The aqueous mixture was extracted three times with 100-ml portions of ether. The organic layers were combined, washed with water, saturated sodium bicarbonate, and again with water, dried over sodium sulfate and concentrated to give 50 g (54%) of pale yellow oil.

2-(3',4'-dichlorophenyl)-phenol --
To a flask fitted with a gas bubbler were added 21.3 g (0.1 mole) of 2-phenylphenylacetate in 300 ml of carbon tetrachloride and a trace of

iodine. To this solution 15.6 g (0.22 mole) of chlorine was slowly bubbled over a period of 2.5 hours. Stirring was continued for two hours, and the solvent was removed.

This crude material was placed into a 500-ml round-bottom flask, and 150 ml of ethanol, 150 ml of water, and 50 g of potassium hydroxide were added. The mixture was refluxed for 15 minutes, allowed to cool, poured into 100 ml of water and acidified, and the oil was separated. The oil was extracted with ether and dried in the usual manner. A silica-gel column was prepared (100 g), and the material was eluted in 10 carbon tetrachloride in petroleum ether (30-60). The first fraction off the column was crystalline (mp 144-145°), 11.5 g (48% yield).

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SECTION 6

BIOLOGICAL STUDIES

STRUCTURE-TOXICITY CORRELATIONS OF PHENOLIC COMPOUNDS DETERMINED WITH *DAPHNIA MAGNA*

Results and Discussion

To investigate the environmental impact of the products derived from the aqueous chlorination and ozonation of organic compounds known to exist in waste effluents, a rapid and reliable bioassay was needed to determine which compounds were highly toxic. *Daphnia magna* was chosen for the screening procedure because this animal is relatively easy to rear and to manipulate, and it is responsive to added toxicants[1,6] such as to be considered generally representative of aquatic invertebrates.

Phenols were studied because of their frequent appearance in effluents and their availability as pure samples bearing systematic structural variations. In addition, a significant amount of mechanistic and toxicity data is available on phenol itself[7-12]. Phenols assume added significance when it is recognized that all the compounds listed in Table 11 have the ability to incorporate chlorine over a wide range of pH and concentration.[13]

The structure-activity correlation was investigated by using the procedures developed by Hansch.[14] This method[15-19] attempts to correlate the biological response (BR) with two parameters, the relative partition coefficient π and the Hammett electronic substituent constant σ. The partition coefficients are evaluated by using an n-octanol-water system, and π is defined as $\log P_x - \log P_H$, where P_H is the partition coefficient for phenol itself, and P_x is the partition coefficient for a derivative.

The general form of the Hansch equation is shown below in Eq. (1).

$$BR = a\pi^2 + b\pi\sigma_0 + c\pi\sigma_0^2 + d\sigma + e \qquad (1)$$

One can expect to see simplified versions of Eq. (1) depending on the relative importance of the parameters π and σ and the constant σ_0. These modifications are given in Eq. (2) through (6).

$$BR = k_1\pi + k_2 \qquad (2)$$

$$BR = k_1\pi^2 + k_2\pi + k_3 \qquad (3)$$

$$BR = k_1\sigma + k_2 \qquad (4)$$

$$BR = k_1\pi + k_2\sigma + k_3 \qquad (5)$$

Figure 4. Log of the percent survivors vs. time, using p-cresol.

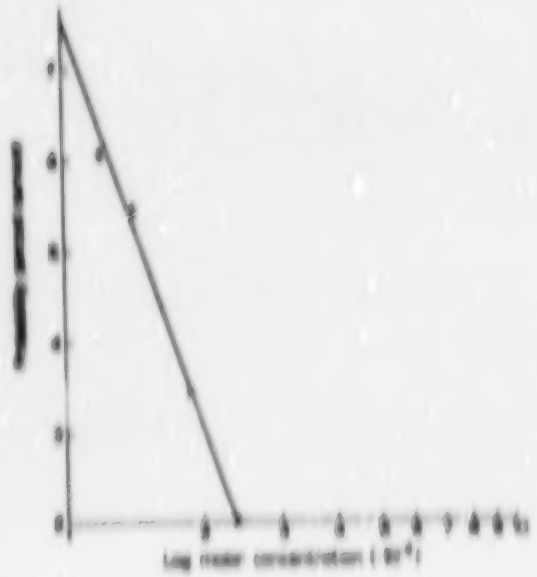

Figure 5. Probability of survival vs. log of the molar concentration, using o-cresol.

TABLE 11. TOXICITY OF SUBSTITUTED PHENOLS TO DAPHNIA MAGNA

Function	π_x	π_y	ΣF	ΣR	log 1/c (calcd.	log 1/c (observed	Δlog 1/c	c (observed)
3-Methoxy	0.12	0.10	0.26	-0.01	3.576	3.680	-0.064	$3.31 \cdot 10^{-4}$
3-Methoxy	-0.27	0.33	0.26	-0.01	3.634	3.680	0.046	$2.09 \cdot 10^{-4}$
4-Methoxy	-0.27	0.48	0.26	-0.13	3.807	3.709	-0.198	$1.93 \cdot 10^{-4}$
3-Methyl	-0.17	0.49	0.24	-0.13	3.913	3.833	-0.077	$1.48 \cdot 10^{-4}$
H	0.00	0.00	0.00	0.00	3.731	3.904	0.173	$1.01 \cdot 10^{-4}$
3,5-Dimethyl	-0.34	0.69	0.48	-0.26	4.042	4.010	-0.032	$9.30 \cdot 10^{-5}$
4-Nitro	1.23	0.90	0.67	0.16	4.287	4.219	-0.168	$6.04 \cdot 10^{-5}$
3-Chloro	0.23	0.69	0.41	-0.13	4.167	4.238	0.071	$5.78 \cdot 10^{-5}$
4-Chloro	0.23	0.93	0.41	-0.13	4.287	4.426	0.139	$3.73 \cdot 10^{-5}$
4-Bromo	0.23	1.13	0.44	-0.17	4.385	4.453	0.074	$3.54 \cdot 10^{-5}$
3,5-Dinitro	1.03	0.83	1.34	0.33	4.972	4.991	0.019	$1.02 \cdot 10^{-5}$
4-Phenyl	2.01	1.91	0.08	-0.08	4.973	4.647	-0.326	$2.13 \cdot 10^{-5}$
3,4-Dichloro	0.46	1.62	0.83	-0.31	4.710	4.795	0.085	$1.60 \cdot 10^{-5}$
3,4,5-Tribromo	0.81	3.91	1.33	-0.31	5.461	5.403	-0.058	$3.91 \cdot 10^{-6}$

$$BR = k_1\sigma^2 + k_2\sigma + k_3\sigma + k_4 \qquad (6)$$

Equation 4 considers the unlikely case where there is no dependency on π. A dependency on σ only would suggest a situation more easily visualized in an *in vitro* system rather than an *in vivo* one.[16]

Hansch *et al.*[20] recently reported using a new set of parameters, F and R, which attempted to separate the inductive (F) and resonance (R) components of the substituents. Because of the method by which F and R have been derived (i.e., from σ_m and σ_p) and the demonstration of the additive nature of σ for use in structure-activity correlations,[9] it appears reasonable to assume that F and R are additive. We utilized this argument and included the following two additional equations in the attempted correlation[21,22,23]

$$BR = c_1\pi + c_2F + c_3R + c_4 \qquad (7)$$

$$BR = c_1\pi^2 + c_2\pi + c_3F + c_4R + c_5 \qquad (8)$$

The data from the phenolic series in Table II were evaluated by using the seven possible correlations [Eq. (2) through (8)]. The results are listed below, where r is the correlation coefficient and c is the molar concentration at LC_{50}. The best correlations were obtained in Eq. (7) and (8), which have both F and R dependency.

	r	
$\log 1/c = 0.527\ \pi + 3.796$	0.831	(9)
$\log 1/c = 0.089\ \pi^2 + 0.371\ \pi + 3.851$	0.835	(10)
$\log 1/c = 0.339\ \sigma + 4.129$	0.480	(11)
$\log 1/c = 0.173\ \sigma + 0.282\ \sigma + 3.914$	0.905	(12)
$\log 1/c = 0.062\ \sigma^2 + 0.095\ \sigma + 0.364\ \sigma + 3.613$	0.965	(13)
$\log 1/c = 0.500\ \pi + 0.453\ F + 0.637\ R + 3.731$	0.978	(14)
$\log 1/c = -0.028\ \pi^2 + 0.567\ \pi + 0.480\ F + 0.607\ R + 3.695$	0.978	(15)

The error analysis showed that Eq. (14) represented the best correlation as the t values observed for the coefficients of the π^2 terms in Eq. (10), (13) and (15) indicated that these parameters added little or nothing to the correlation. The apparent improved correlation of Eq. (13) or (12) is, therefore, not significant.

It must be emphasized that a correlation of this type is likely only if a series is picked in which the mode of death remains constant, and at best these correlations will only indicate trends. It can only be assumed that extreme deviations correspond to a change in the mechanism of toxic action.

Increasing halogen substitution (i.e., enhanced lipophilic nature) in the phenol resulted in increased toxicity in agreement with the π, F, and R dependency. This observation is particularly significant in the

current evaluation of the relative merits of chlorination and ozonation as techniques for wastewater renovation and the previously observed incorporation of carbon-bound chlorine by a variety of phenolic systems under disinfection conditions.

Experimental

Three or more tests (four dose levels for each test) were run for each compound under investigation. The data treatment was completely computerized using least squares. However, for clarity it will be explained as if each test were plotted by hand.

For each compound a graph was prepared plotting log percentage survivors vs. time in hours.[24] From this plot the percentage survivors for each dose level at 48 hours could be determined. This procedure tends to average the results and increase the repeatability factor involved with biological testing. In addition, all values, from a 24-hour to a 96-hour LC_{50} can be calculated by using this one set of data. The 48-hour percentage survival figures were converted to probit values by using probit transformation tables[25]. These values were plotted vs. log molar concentration. The log LC_{50} molar concentration can then be found by observing the log molar concentration value that corresponds to a probit value of 5. The probit values and log molar concentration values were introduced into a least squares computer program which calculated the LC_{50} and also gave the correlation of the line to the data points.

This method for determining the 48-hours LC_{50} value has the advantage of a four-day observation period. The inconsistencies that arise when the animals are counted only once (at 48 hours) are, therefore, averaged out, and greater reproducibility of the data results. An example of this process is illustrated for p-cresol (Figures 4 and 5).

As a measure of the reliability of the screening procedure, a structure-activity correlation was attempted for a series of phenols. Rather than predict absolute values, we hoped to recognize trends within a given structural series, as has been done with correlations successfully carried out in pharmaceutical drug design.[18,26,27]

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Results and Discussion

The availability of a rapid and accurate technique for the determination of partition coefficients (or their equivalent) became desirable when it was observed that the partition coefficient between n-octanol and water ($P_{o/w}$) was dominant in the successful Hansch correlation of phenol toxicity to aquatic species.[1] This concern has led to the development of a method that uses a permanently bonded long-chain alkyl packing in a high-pressure liquid chromatographic system and subsequently relates the capacity factor k' = $(t_s-t_0)/t_0$, i.e., the net retention time relative to the nonadsorbed time, to the partition coefficient $P_{o/w}$.

It is well recognized that a separation (i.e., a difference in retention volume) in "reverse-phase" chromatography depends upon the partitioning characteristics of the solute between the mobile phase and the stationary phase as represented by the value of the partition coefficient. To evaluate the elution data in the current study, it was, therefore, necessary to consider the relative number of moles of eluting solvent. However, the use of mole percent resulted in a significant increase in the deviation from linearity from that previously observed in the analysis of thin-layer partition data[2] when R_M [R_M = log (1/R_f-1)] was plotted against volume percent.

For example, Figures 6 and 7 contain plots of log k' vs. volume percent[3] (sigma y = 0.012) and log k' vs. mole percent (sigma y = 0.035), respectively, for a representative compound (o-chlorophenol). It was subsequently found that the linearity could be maintained and substantially extended for all the compounds studied by plotting log ($1/k' + 1$) vs. mole percent (e.g., o-chlorophenol, Figure 7, sigma y = 0.009). This change is valid as the correlations of R_M and k' are made over a range of percentages, and the modification is, therefore, only from one empirical relationship to another.

The correlations of log k' to log P and π (π = log $P_{substituted}$ - log P_{parent}) to π (π = log $k'_{substituted}$ - log k'_{parent}) for some phenols and anilines are contained, along with their corresponding residuals, in Tables 13 and 14. The coefficients obtained from the individual regression analyses are found to be quite satisfactory. However, when the results from the two families of compounds are combined, the correlation of log P

to log k' decreases (r = 0.86). Although this is an acceptable value for a regression analysis of this type, it indicates that P or k', or both, reflect more than just lipophilic character.

PHENOLS:

$$\log P = A \log k' + C \qquad r = 0.96$$

$$\pi = M_\pi + C \qquad r = 0.96$$

ANILINES:

$$\log P = A \log k' + C \qquad r = 0.97$$

$$\pi = M_\pi + C \qquad r = 0.97$$

Related to these observations is the work of Collander[3,4], which has shown that although any two alcohol:water systems will provide a linear relationship between log P values, it is not possible to extend the correlation over a wide range of solvent types (alcohols, ester, ketones, halogenated hydrocarbons). The successful correlation in the present study, therefore, indicates that there is a good approximation of the solvent forces in C-18 Bondapak/Acetone-H_2O to those of alcohol/water systems or that the chemical potential of either the lipophilic (π_l) or hydrophilic (π_h) components of the solute remains constant.

$$\log P = \frac{M_{\pi l} + J_{\pi h}}{2.301}$$

In addition, although neither the octanol/water system nor the Bondapak C-18/Acetone-H_2O chromatographic system can be construed to be structurally representative of a biological membrane, the somewhat comparable results upon substitution of π for π in a Hansch-type biological correlation[5-9] (Table 12) indicate the predictive powers of k' and P in evaluating the ability of an organic molecule to pass through biological tissue.[10]

$$\log 1/c = M_\pi + C \qquad r = 0.78$$

$$\log 1/c = M_\pi + C \qquad r = 0.88$$

Experimental

The separations were performed on 2-1/8" x 2' Bondapak C-18/Porasil B columns that were mounted in a Waters Associates ALC 202 (refractive index detector). The various mole percentages of distilled water and acetone (HCP-ACS grade) were eluted at 24-26°C and a flow rate of 0.9-1.0 ml/min.

TABLE 12. COMPARISON OF OBSERVED AND CALCULATED BIOLOGICAL RESPONSES FOR DAPHNIA MAGNA TOXICITY IN THE PRESENCE OF SUBSTITUTED PHENOLS.

Compound	Observed log (1/c)	Calculated log (1/c) from K	Calculated log (1/c) from π
Phenol	3.901	3.769	3.647
3-Methoxyphenol	3.480	3.210	3.715
4-Nitrophenol	4.210	3.845	3.952
4-Methylphenol	3.709	3.996	3.972
2-Methylphenol	3.835	4.073	3.979
2 -Chlorophenol	4.230	4.065	4.114
2,6 Dimethylphenol	4.036	4.764	4.243
4-Chlorophenol	4.426	4.244	4.277
4-Bromophenol	4.463	4.356	4.412

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TABLE 13. THE CORRELATION OF LOG k' TO LOG P AND σ TO σ FOR SUBSTITUTED ANILINES

Substituent	Log P	Log k'	Calc. log P	σ	σ	Calc. σ
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE 14. THE CORRELATION OF LOG k' TO LOG P AND σ TO σ FOR SUBSTITUTED PHENOLS

Substituent	Log P	Log k'	Calc. log P	σ	σ	Calc. σ
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

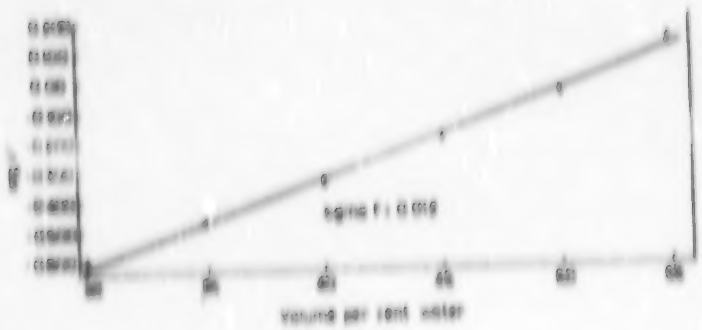

Figure 6. Plot of log k' vs. volume per cent of water for o-chlorophenol.

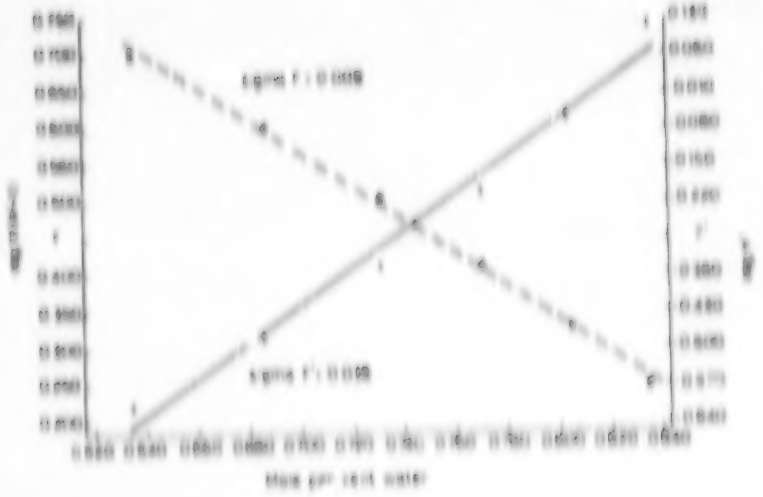

Figure 7. Plots of log [1/(k' + 1)] and k' vs. mole per cent of water for o-chlorophenol.

Results and Discussion

The biological oxygen demand (BOD) reduction in chlorinated wastewater is a well-recognized phenomenon.[1,2] However, the recent studies of Zaloum and Murphy[2] suggest that the dominant factor in BOD reduction is the significant decrease in the microbial populations of the chlorinated wastewater BOD test solutions when compared with the unchlorinated samples. The results are also consistent with the conclusion that BOD reduction is not some inherent character of the chemical process itself. Therefore, the observations were not associated with the possible oxidative conversion of the soluble organics to some chlorinated progeny that might, in turn, prove to be bioresistant or toxic, or both. However, this latter conclusion was not reconciled with substantial previous evidence that, based upon the initial chlorine dose, approximately 1-2% of the available chlorine can be accounted for as "second-order" chlororganics.[3,4,5]

Non-heterocyclic aromatics found in wastes subjected to chlorination include phenols, benzoic acids, and, to a lesser extent, anilines, benzyl-alcohols and polynuclear aromatics.[3,6] Chlorinated examples of several of these compound types have been found after chlorine disinfection.[3] Among the compounds present, phenolic material is not only the most ubiquitous, but also the most vulnerable to chlorine incorporation over the entire range of possible pH values.[7] The presence of such chlorophenolic compounds may indeed have an effect upon possible degradative processes, but proof would depend upon an experimental design that would not only provide comparable initial microbial populations, but also provide the opportunity to compare any observation to a situation when no chlorination had occurred.

Results of BOD tests on dosed chlorinated phenols, benzoic acids and anilines were evaluated against the parent compound (i.e., phenol, benzoic acid and aniline) and against a control. The various compounds were compared on a molar basis and, relative to the internal glucose-glutamic acid standard, possessed the same initial microbial content. The results clearly indicate that parent systems differ in their vulnerability during the test period and that the "chloroproducts" have diminished degradability relative to the parent. Moreover, the decrease in BOD relative to the internal standard suggests that the "second-order" chlororganics in sufficiently high concentration can have an adverse effect on the microbial community.

The results from the present investigation when compared to those of Zaloum and Murphy demonstrate that the BOD test will not differentiate the significant increase of the bioresistance of the chlororganics when they are present at sufficiently low levels. However, considering the apparent toxic effects of some of the chlorinated materials at the ppm level, it would be surprising if these materials are not contributing to the observed decrease in the overall microbial population observed by these investigators. An overall summary of the investigation is provided in Tables 15-18.

[illegible]

[illegible]

[illegible]

TABLE 15. SUMMARY OF BOD RESULTS FOR PHENOLS (CONTINUED)
(0-5 DAYS)

Sample†	0 hr mg/l	24 hr mg/l	48 hr mg/l	72 hr mg/l	96 hr mg/l	120 hr mg/l
GGA* + seed	0	0	129	145	175	202
GGA + seed + phenol	0	0	175	190	241	274
GGA + seed + o-chlorophenol	0	5	116	140	170	203
GGA + seed + p-chlorophenol	0	12	126	166	194	217
GGA + seed + 2,4-dichlorophenol	0	10	129	129	174	200

Sample†	0 hr	24 hr	48 hr	72 hr	96 hr	120 hr
GGA* + seed	0	0	134	181	235	264
GGA + seed + phenol	0	13	223	287	345	385
GGA + seed + cresol	0	10	209	274	359	384
GGA + seed + 2-chloro-5-methyl-phenol	0	0	170	189	268	272
GGA + seed + 4-chloro-3-methyl-phenol	0	0	0	40	144	161

† 1×10^{-3}M in phenol or substituted phenol.

* GGA = Glucose-Glutamic acid; 5 ml of GGA per DO bottle was used. The BOD values were calculated using 5 mls of GGA as the sample volume. One milliliter of settled (24 hrs) fresh sewage seed per one liter dilution water was used. The incubator temperature was 20°C ±1°C throughout the incubation period.

TABLE 16. SUMMARY OF BOD RESULTS FOR BENZOIC ACID

Test #	Benzoic Acid 5	Benzoic Acid 10	p-Chloro Benzoic Acid 5	p-Chloro Benzoic Acid 10
2 x 10-6M (0.24 ppm)	250	230		
6 x 10-6M (0.73 ppm)	254	244		
1 x 10-5M (1.22 ppm)	246	246		
2 x 10-6M (0.31 ppm)			142	166
6 x 10-6M (0.94 ppm)			142	152
1 x 10-5M (1.57 ppm)			139	156

Blank: Glucose-Glutamic Acid + seed = 164 (5 day); 168 (10 day)

TABLE 17. SUMMARY OF BOD RESULTS OF ANILINES

Test #	Aniline 5	Aniline 10	Aniline 20	p-Chloro Aniline 5	p-Chloro Aniline 10	p-Chloro Aniline 20
2 x 10-6M (0.19 ppm)	147	179	178			
6 x 10-6M (0.56 ppm)	140	153	121			
1 x 10-5M (0.93 ppm)	160	161	141			
2 x 10-6M (1.28 ppm)				131	140	120
1 x 10-5M (12.8 ppm)				94	140	114

Blank: Glucose-Glutamic Acid + seed = 155 (5 day); 161 (10 day); 193 (20 day)

1. Barnhart, E. L. and G. R. Campbell, "Effect of Chlorination on Selected Organic Chemicals," U. S. Environmental Protection Agency, Washington, D. C., Publication No. 12020 EXG03/72.

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3. Jolley, R. L., "Chlorination Effects on Organic Constituents in Effluents from Domestic Sanitary Sewage Treatment Plants," Ph.D. Dissertation, University of Tennessee, Knoxville, TN, 1973.

4. Jolley, R. L., Environ. Lett., 7, 321 (1974).

5. Pitts, W. M., Jr., R. L. Jolley and C. D. Scott, Environ. Sci. Tech., 9, 1068 (1975).

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APPENDIX A

SUMMARY OF LITERATURE ON *DAPHNIA MAGNA* TOXICITY

Acetic Acid
- LD_{50}2.:47 ppm (1,SRW**)
- max LD_{0} = 150 ppm* (3)

Acetone
- LD_{50}24-10 ppm (1,RDW)
- LD_{50}48-10 ppm (1,RDW)
- max LD_{0} = 9280 ppm (3)

Aldrin
- LD_{100}50-29.8 ppb (8)
- LD_{50}24-30.0 ppb (4)
  - 50-29.8 ppb (4)
- LD_{50}26-30.0 ppb (5)

Aluminum
- LD_{50}48-3.9 ppm (8,WOF)

Aluminum Sulfate
- max LD_{0} = 136 ppm (3)

Aluminum Ammonium Sulfate
- max LD_{0} = 190 ppm (3)

Aluminum Potassium Sulfate
- max LD_{0} = 206 ppm (3)

Ammonium Chloride
- LD_{50}24-202 ppm (1,ULW)
- LD_{50}48-161 ppm (ULW)
- LD_{50}72-67 ppm (ULW)
- LD_{50}96-50 ppm (ULW)
- LD_{50}100-139 ppm (SRW)
- max LD_{0} = <134 ppm (3)

Ammonium Hydroxide
- LD_{50}25-60 ppm (1,SRW)
- LD_{50}50-32 ppm (1,SRW)
- LD_{50}100-20 ppm (1,SRW)
- max LD_{0} = <8.75 ppm (3)

Ammonium Sulfate
- LD_{50}25-423 ppm (1,SRW)
- LD_{50}50-433 ppm (1,SRW)
- LD_{50}100-292 ppm (1,SRW)
- max LD_{0} = <106 ppm (3)

Ammonium Sulfite
- LD_{50}25-299 ppm (1,SRW)
- LD_{50}50-273 ppm (SRW)
- LD_{50}100-203 ppm (SRW)

Aniline max LD_{0} = 279 ppm (3)
- LD_{50}48-0.34 ppm (8)

o-Anisidine LD_{50}48-4.71 ppm (8)

p-Anisidine LD_{50}48-0.73 ppm (8)

Anisole LD_{50}48-140 ppm (7)

Anisonitrile LD_{50}48-33 ppm (7)

Arsenite LD_{50}26-69.0 ppb (8)

Arsenic LD_{50}48-7.4 ppm (8,WOF)

Atrazine LD_{50}26-3.60 ppm (5)

* Maximum allowable for zero toxicity over infinite time

**SRW - Standard research water
ULW - University lake water
RDW - Reference dilution water
WOF - Without food in test

Azinphosmethyl (Guthion)
LD_{50}26-185 ppt (6)
Barium LD_{50}48-14.5 ppm (6,WOF)
Barium Chloride
max LD_{0} = <83 ppm (3)
Benzene LD_{50}48-410 ppm (7)
Benzoic Acid max LD_{0} = 146 ppm (3)
Benzonitrile LD_{50}48-250 ppm (7)
Benzylalcohol LD_{50}48-250 ppm (7)
o-Bromoaniline LD_{50}48-0.20 ppm (8)
p-Bromoaniline LD_{50}48-28 ppm (7)
p-Bromophenol LD_{50}48-5.9 ppm (7)
Butyric Acid LD_{50}48-61 ppm (1,SRW)
Cadmium LD_{50}48-65 ppb (6,WOF)
Calcium LD_{50}48-464 ppm (6)
Calcium Chloride
LD_{50}26-3526 ppm (1,SRW)
LD_{50}[illegible]-3005 ppm (1,SRW)
LD_{50}24-1838 ppm (1,ULW)
LD_{50}48-759 ppm (1,ULW)
LD_{50}72-759 ppm (1,ULW)
LD_{50}100-649 ppm (1,ULW)
max LD_{0} = 1332 ppm (3)
Captan LD_{50}26-1.30 ppm (5)
Carbophenothion LD_{50}26-9 ppt (5)
Carvone LD_{50}48-44 ppm (7)
o-Chloroaniline LD_{50}48-0.61 ppm (8)
p-Chloroaniline LD_{50}48-0.13 ppm (8)
Chlorobenzene LD_{50}48-80 ppm (7)
Chlorobenzilate
LD_{100}50-1.4 ppm (2)
LD_{50}26-1.45 ppm (5)
o-Chlorophenol LD_{50}48-7.6 ppm (7)
p-Chlorophenol LD_{50}48-4.7 ppm (7)
Chlorthion LD_{50}26-4.5 ppb (5)
Chromic Acid max LD_{0} = <<0.6 (3)
Chromic Sulfate
LD_{50}24-100 ppb (1,ULW)
LD_{50}48-30 ppb (1,ULW)
Citric Acid max LD_{0} = 183 ppm (3)
Cobalt LD_{50}48-1.62 ppm (6)
Cobaltous Chloride
max LD_{0} = <<28 ppm (3)
Copper LD_{50}48-65 ppb (6)
Coumaphos LD_{50}26-100 ppt (5)
Capric Chloride max LD_{0} = 80 ppb (3)
Cupric Sulfate max LD_{0} = 96 ppb (3)
Cyclethrin LD_{50}26-55.0 ppb (5)
Dalapon (Acid) LD_{50}26-6.00 ppm (5)
DDD (TDE) LD_{50}24-4.6 ppb (4)
DDT LD_{100}50-1.4 ppb (2)
LD_{50}24-4.4 ppb (4)
50-1.4 ppb (4)
LD_{50}26-4.4 ppb (5)
Demeton LD_{50}26-6.0 ppb (5)
Diazinon
LD_{100}50-4.3 ppb (2)
LD_{50}50-4.3 ppb (4)
Dicapthon LD_{50}26-4.1 ppb (5)
Dichlone LD_{50}26-26.0 ppb (5)
2,4-Dichloroaniline LD_{50}48-2.93 ppm (8)
2,5-Dichloroaniline LD_{50}48-2.94 ppm (8)
3,4-Dichloroaniline LD_{50}48-0.32 ppm (8)
2,4-Dichloro-6-(o-Chloroaniline)-S-Triazine LD_{50}26-490 ppb (5)
2,4-Dichlorophenol LD_{50}48-2.6 ppm (7)
Dinofol LD_{50}26-390 ppb (4)
Dieldrin
LD_{100}50-330 ppb (2)

LD_{50}24-740 ppb (4)
50-330 ppb (4)
LD_{50}26-740 ppb (5)

Dilan LD_{50}26-21.0 ppb (5)

Dimethoate LD_{50}26-2.50 ppm (5)

m-Dimethoxybenzene
LD_{50}48-98 ppm (7)

2,6-Dimethylphenol
LD_{50}48-11 ppm (7)

Dimite LD_{50}26-290 ppb (5)

2,4-Dinitroaniline
LD_{50}18-10.7 ppm (8)

2,4-Dinitrophenol
LD_{50}48-4.6 ppm (7)

Dioxathion LD_{50}26-330 ppt (5)

Dipterex LD_{50}50-120 ppt (6)

Dodine LD_{50}26-58.0 ppb (5)

Dowco 109 LD_{50}26-400 ppb (5)

Endosulfan LD_{50}24-240 ppb (5)

Endrin LD_{100}50-352 ppb (2)
LD_{50}26-900 ppb (5)
LD_{50}24-900 ppb (4)
50-352 ppb (4)

EPN
Phenylphosphonothioic acid o-ethyl o-p-nitrophenyl
LD_{100}50-100 ppt (2)
LD_{50}26-110 ppt (5)

Ethion LD_{50}26-72 ppt (5)

Ethyl alcohol
max LD_{50} = 10400 ppm (3)

Ethyl N,N-dipropyl-thiocarbamate
LD_{50}26-1.70 ppm (5)

Ethyl parathion
LD_{50}24-600 ppt (4)
50-600 ppt

Farban LD_{50}26-65.0 ppb (5)

Ferric Chloride
LD_{50}25-36 ppm (1,5RW)
LD_{50}50-21 ppm (1,5RW)
LD_{50}100-15 ppm (1,5RW)
max LD_{50} = 130 ppm (3)

Ferrous Sulfate
max LD_{50} = <152 ppm (3)

Formaldehyde
LD_{50}24-<100<1,000 ppm (1 RDW)

Glyodin LD_{50}26-300 ppb (5)

Guaiacol LD_{50}48-26 ppm (7)

Heptachlor
LD_{100}50-57.7 ppb (2)
LD_{50}24-52.6 ppb (4)
50-57.7 ppb
LD_{50}26-52.0 ppb (5)

Heptachlor Epoxide
LD_{50}26-120 ppb (5)

Hydrochloric Acid
max LD_{50} = 82 ppm (3)

Iron LD_{50}48-9.6 ppm (6)

Iso-amyl Alcohol
max LD_{50} = 881 ppm (3)

Isolan LD_{50}26-12.5 ppb (5)

Isopulegal LD_{50}48-74 ppm (7)

Lactic Acid max LD_{50} = 243 ppm (3)

Lead LD_{50}48-450 ppb (6)

Limonene LD_{50}48-6.1 ppm (7)

Lindane LD_{50}50-1,100 ppm (4)
LD_{50}26-1.25 ppm (5)

Magnesium LD_{50}48-322 ppm (6)

Magnesium Chloride
LD_{50}25-3391 ppm (1,5RW)

LD_{50}50-3699 ppm (SRW)
LD_{50}100-3484 ppm (SRW)

Magnesium Sulfate
LD_{50}24-963 ppm (1,ULW)
LD_{50}48-929 ppm (ULW)
LD_{50}72-861 ppm (ULW)
LD_{50}96-788 ppm (ULW)
LD_{50}96-3803 ppm (SRW)

Malathion LD_{100}50-900 ppt (2)
LD_{50}24-900 ppt (4)
50-900 ppt
LD_{50}26-900 ppt (5)

Manganese LD_{50}48-9.8 ppm (6,WOF)

Mercury LD_{50}48-5 ppb (6,WOF)

p-Methoxybenzyl alcohol
LD_{50}48-130 ppm (7)

Methoxychlor LD_{100}50-3.6 ppb (2)
LD_{50}24-3.7 ppb (4)
50-3.6 ppb
LD_{50}26-3.7 ppb (5)

m-Methoxyphenol LD_{50}48-41 ppm (7)

p-Methoxyphenol LD_{50}48-2.6 ppm (7)

Methyl Alcohol
max LD_{50} = 32000 ppm (3)

Methyl parathion
LD_{50}26-4.8 ppb (5)

2-Methylphenol LD_{50}48-16 ppm (7)

3-Methylphenol LD_{50}48-28 ppm (7)

4-Methylphenol LD_{50}48-22 ppm (7)

Naled LD_{50}26-360 ppt (5)

Nemacide (O-2,4-dichlorophenyl) (O,O-diethyl phosphorothioate)
LD_{50}26-1.1 ppb (5)

Nickel LD_{50}48-1.12 ppm (6)

Nitric Acid max LD_{50} = 107 ppm (3)

o-Nitroaniline LD_{50}48-12.0 ppm (8)

m-Nitroaniline LD_{50}48-2.71 ppm (8)

p-Nitroaniline LD_{50}48-13.8 ppm (8)

p-Nitrobenzole LD_{50}48-12 ppm (7)

p-Nitrophenol LD_{50}48-8.2 ppm (7)

Oxalic Acid max LD_{50} = 95 ppm (3)

Parathion LD_{100}50-900 ppt (2)
LD_{50}26-900 ppt (5)

Perthane LD_{50}26-9.4 ppb (5)

Phenol D. magna
LD_{50}24-100 ppm (1,ROW)
LD_{50}48-100 ppm (1,ROW)
D. magna (young)
LD_{50}25-17 ppm (1,SRW)
LD_{50}50-7 ppm (1,SRW)
D. magna (adult)
LD_{50}25-61 ppm (1,SRW)
LD_{50}21 ppm (1,SRW)
max LD_{50} = 94 ppm (3)
LD_{50}48-12 ppm (7)

p-Phenylphenol LD_{50}48-3.6 ppm (7)

Phorate LD_{50}26-2.2 ppb (5)

Phosphamidon
LD_{50}50-12.5 ppb (4)
LD_{50}26-4.0 ppb (5)

Phostex LD_{50}26-16.1 ppb (5)

Potassium LD_{50}48-166 ppm

Potassium Chloride
LD_{50}100-670 ppm (1,SRW)
LD_{50}24-343 ppm (1,ULW)
48-337 ppm (1,ULW)
72-117 ppm (1,ULW)
96-29 ppm (1,ULW)
max LD_{50} = 373 ppm (3)

Potassium Cyanide
LD_{50}24-2 ppm (1,ULW)
48-2 ppm (1,ULW)
72-700 ppb (1,ULW)
96-400 ppb (1,ULW)

Potassium Dichromate
LD_{50}100-400 ppb (1,SRW)
max LD_{0} = <<600 ppb (3)

Potassium Ferricyanide
LD_{50}24-905 ppm (1,ULW)
48-549 ppm (1,ULW)
72-600 ppb (1,ULW)
96-100 ppb (1,ULW)

Potassium Nitrate
LD_{50}24-490 ppm (1,ULW)
48-490 ppm (1,ULW)
72-226 ppm (1,ULW)
96-39 ppm (1,ULW)
LD_{50}100-900 ppm (1,SRW)

Potassium Permanganate
max LD_{0} = 630 ppb (3)

Prometone LD_{50}26-35.0 ppm (5)

Propionic Acid
LD_{50}48-50 ppm (1,SRW)

Pyridine LD_{50}24-2,114 ppm (1,ROW)
48-944 ppm (1)

Ronnel LD_{50}26-1.8 ppb (5)

Sodium LD_{50}48-1820 ppm (6)

Sodium Anthraquinone
alpha-sulfonate
LD_{50}24-186 ppm (1,ULW)
48-186 ppm (1,ULW)
72-186 ppm (1,ULW)
96-50 ppm (1,ULW)
LD_{50}100-12 ppm (SRW)

Sodium Arsenate
max LD_{0} = 31 ppm (3)

Sodium Bicarbonate
max LD_{0} = 4200 ppm (3)

Sodium Bisulfite
D. magna - young
LD_{50}25-116 ppm (1,SRW)
50-81 ppm (1,SRW)
D. magna - adult
LD_{50}96-102 ppm (1,SRW)

Sodium Bisulfite
D. magna - adult
LD_{50}24-171 ppm (1,ULW)
48-119 ppm (1,ULW)
72-97 ppm (1,ULW)
96-82 ppm (1,ULW)

Sodium p-bromobenzene sulfonate
LD_{50}24-2347 ppm (1,ULW)
48-1943 ppm (1,ULW)
72-971 ppm (1,ULW)
96-809 ppm (1,ULW)
LD_{50}100-923 ppm (1,SRW)

Sodium Butyl Sulfonate
LD_{50}24-8,000 ppm (1,ULW)
48-8,000 ppm (1,ULW)
72-8,400 ppm (1,ULW)
96-8,700 ppm (1,ULW)

Sodium Carbonate
LD_{50}24-337 ppm (1,ULW)
48-265 ppm (1,ULW)
LD_{50}25-607 ppm (1,SRW)
48-565 ppm (1,SRW)
96-524 ppm (1,SRW)
max LD_{0} = 424 ppm (3)

Sodium Chloride

LD_{50}25-6447 ppm (1,SRW)
50-5874 ppm (1,SRW)
100-3114 ppm (1,SRW)
LD_{50}24-3412 ppm (1,ULW)
48-3310 ppm (1,ULW)
max LD_{50} = 6143 ppm (3)

Sodium p-chlorobenzene sulfonate

LD_{50}24-8600 ppm (1,ULW)
48-7659 ppm (1,ULW)
72-3964 ppm (1,ULW)
96-2150 ppm (1,ULW)
LD_{50}100-2394 ppm (1,SRW)

Sodium 2-chlorotoluene-5-sulfonate

D. magna - young
LD_{50}25-800 ppb (1,SRW)
50-600 ppb (1,SRW)
100-400 ppb (1,SRW)
D. magna - adult
LD_{50}25-3.5 ppm (1,SRW)
50-1.3 ppm (1,SRW)

Sodium 2,5-dichlorobenzene sulfonate

LD_{50}24-4931 ppm (1,ULW)
48-6931 ppm (1,ULW)
72-2490 ppm (1,ULW)
96-938 ppm (1,ULW)
LD_{50}100-1468 ppm (1,SRW)

Sodium Dichromate

LD_{50}24-22 ppm (1,ULW)
48-10 ppm (1,ULW)

Sodium Hydroxide

max LD_{50} = 240 (3)

Sodium Pathyldithiocarbamate

LD_{50}26-330 ppb (5)

Sodium mono-hydrogen phosphate

LD_{50}25-1164 ppm (1,SRW)
50-1089 ppm (1,SRW)
100-426 ppm (1,SRW)

Sodium Nitrate

LD_{50}24-5980 ppm (1,ULW)
48-3581 ppm (1,ULW)
72-2125 ppm (1,ULW)
96-665 ppm (1,ULW)
LD_{50}96-665 ppm (1,SRW)
max LD_{50} = 9500 ppm (3)

Sodium m-nitrobenzene sulfonate

LD_{50}24-8665 ppm (1,ULW)
48-8665 ppm (1,ULW)
72-6017 ppm (1,ULW)
96-5067 ppm (1,ULW)
LD_{50}100-2235 ppm (1,SRW)

Sodium 4-nitrochloro-benzene-2-sulfonate

LD_{50}24-1698 ppm (1,ULW)
48-1463 ppm (1,ULW)
72-948 ppm (1,ULW)
96-948 ppm (1,ULW)
LD_{50}96-1474 ppm (1,SRW)

Sodium p-phenolsulfonate

LD_{50}24-13510 ppm (1,ULW
48-13510 ppm (1,ULW)
72-3594 ppm (1,ULW)
96-1471 ppm (1,ULW)

Sodium Phosphate

LD_{50}25-237 ppm (1,SRW)
50-177 ppm (1,SRW)

100-126 ppm (1,SRW)

Sodium Pyrophosphate

LD_{50}24-433 ppm (1,ULW)

48-391 ppm (1,ULW)

Sodium Silicate

LD_{50}24-575 ppm (1,ULW)

48-494 ppm (1,ULW)

72-413 ppm (1,ULW)

96-216 ppm (1,ULW)

LD_{50}96-247 ppm (1,SRW)

Sodium Sulfate

D. magna

LD_{50}24-8364 ppm (1,ULW)

48-2564 ppm (1,ULW)

72-725 ppm (1,ULW)

96-630 ppm (1,ULW)

D. magna - adult

LD_{50}96-4547 ppm (1,SRW)

D. magna - young

LD_{50}24-6800 ppm (1,SRW)

48-6100 ppm (1,SRW)

max LD_{50} = 7105 ppm (3)

Sodium Sulfide

LD_{50}25-16 ppm (1,SRW)

50-13 ppm (1,SRW)

100-6 ppm (1,SRW)

Sodium Sulfite

LD_{50}25-299 ppm (1,SRW)

50-273 ppm (1,SRW)

100-203 ppm (1,SRW)

max LD_{50}=3784 ppm (3)

Sodium Thiosulfate

LD_{50}25-2240 ppm (1,SRW)

50-1334 ppm (1,SRW)

100-805 ppm (1,SRW)

Strontium LD_{50}48-125 ppm (6,WOF)

Sulfuric Acid max LD_{50} = 88 ppm (3)

Sulphenone LD_{50}?6-210 ppm (5)

Tannic Acid max LD_{50} = <<26 ppm (3)

Tartaric Acid

max LD_{50} = 135 ppm (3)

TDE LD_{50}26-4.5 ppb (5)

α-Terpineol LD_{50}48-120 ppm (7)

Thanite LD_{50}26-900 ppb (5)

Thiram LD_{50}26-1.30 ppm (5)

Tin LD_{50}48-55 ppm (6)

m-Toluidine LD_{50}48-0.18 ppm (6)

o-Toluidine LD_{50}48-3.52 ppm (6)

Toxaphene LD_{50}26-94.0 ppb (5)

2,4,6-Tribromophenol

LD_{50}48-1.3 ppm (6)

Trichlorfon LD_{50}26-120 ppt (5)

Valeric Acid LD_{50}48-45 ppm (1,SRW)

Xylene LD_{50}24-<100<1,000 ppm (1,ROW)

Zinc LD_{50}48-280 ppb (6)

Zinc Sulfate max LD_{50} = <48 ppm (3)

Ziram LD_{50}26-16.0 ppb (5)

Zineb LD_{50}26-200 ppb (5)

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APPENDIX B
COMPUTER PROGRAM FOR COMPUTATION OF LD_{50}

PROGRAM BIOASSA"

The objective of this program is to allow the computer to calculate the LD_{50} (or any other specified biological response).

This program assumes data has been collected periodically over some given time period (3 or more points). Therefore, the first step is to perform a least squares computation on this data and calculate the required information which will be needed for the second step.

In our case we counted survivors (96 hr statics using Daphnia magna) every 24 hours for 96 hrs, carried out a weighted least squares treatment on the data and from that calculated the expected percent survivors at 48 hours.

The second step in the program incorporates the percent survivors at 48-hour information into another least squares treatment (unweighted) which in turn yields the desired LC_{50} information. In this calculation a least squares treatment is carried out on a plot of molar concentration versus probit percent survivors at 48 hours.

As stated this program uses least squares treatment to analyze the data. The main program has at its disposal two subroutines, one which is an unweighted least squares program and the other is a weighted least squares program.

The weighted program was used in the first step giving the largest weight to the initial point (time zero), while the last point (time final) was given the smallest weight. The reasoning being that at T0 we assumed all animals to be healthy, and at any time after that we can have doubts about the survivors' health and at the end of the test, we are dealing with the super hardy fraction which is not a true representation of the average animal population.

Directions for punching and arranging data cards

The program is designed to accept data in the following format. The first 10 data cards (10F6.3) are probit values which must be read into the computer each time. These values were taken from the literature. Next, data involving one compound at a time is fed into the computer as follows:

FIRST CARD

```
1 2 3 4 | 5 ——→14 | 15 ——→36    column spacing on card
  1 0   | M.W.   | compound name
    A       B          C
```

A. The number of dose levels tested punched in card (I4 format). This example has 10 dose levels.

B. The molecular weight (M. W.) is punched in next (F 10.4 format).

C. The next piece of information on this card is the compound name. spaces 15 to 36 are used for this.

The next card refers to the first dose level tested. This card has the information about the number of data points (A; 6 in this example) and the molar concentration for the dose (B).

SECOND CARD

```
1 2 3 4 | 5 ——→14
    6   | molar conc
    A       B
```

This card stipulates that there are 6 data points; therefore, the next 6 cards contain the bioassay data which was collected. An example for one of these cards is shown. Each card contains time, percent survivors and the weight factor which should be given that particular point (all are F10.4 format).

```
1 ——→10          11 ——→20          21 ——→30
 TIME          % SURVIVORS      WEIGHT FACTOR
```

At this point all the points for one dose level have been entered. This process is repeated for each dose level by punching a card analogous to the second card described above. After all the dose levels and their corresponding data points have been read in, the next compound is entered using a card analogous to the first card described above and the whole process repeated for it. This is repeated until all compounds and data have been entered.

The last card in the data deck must have a zero punched in space 4. This will terminate the program.

```
      PROGRAM BIOPROBY
C     THIS PROGRAM IS DESIGNED TO ANALYZE BIOASSAY DATA AND PRINT OUT THE
C     RESULTANT LC50'S IN THE FOLLOWING MANNER FOR A MAXIMUM OF 30 COMPOUNDS
C
C
C       NAME   NO. OF DOSE        LC50          CORRELATION   SIGMA
C              LEVELS      LOG 1/C   PPM                     (LOG 1/C)
C
C     O-CRESOL     7          1.790   17.22      .994         .029
C                 .
C
C     THIS PROGRAM WILL ALSO SUPPLY RESULTS FROM THE SUBROUTINE CALCULATIONS
C
C             MASK = 40  (IF SUBROUTINE RESULTS WANTED)
C
C             MASK = 44MASK (IF SUBROUTINE RESULTS NOT WANTED)
C
C
C     PROBIT(1200),DUMMY(10,10)   ARE FOR READING IN LITERATURE PROBIT VALUES
C
C     AMW   MOLECULAR WEIGHT
C     PPM   PARTS PER MILLION
C     ANAME COMPOUND NAME
C     TIME  TIME THE DATA WAS TAKEN  (TOTAL ELAPSED TIME)
C     DAPHZA  PER-CENT SURVIVORS
C     FISH    LOG PER-CENT SURVIVORS
C     PROB    IS PARTICULAR PROBIT VALUE TO BE STORED
C     BR      BIOLOGICAL RESPONSE (LOG 1/C)
C     ELC50   LC50
C     RR      CORRELATION OF POINTS TO LINE IN FINAL PROBIT PLOT
C     SIGMAR  STANDARD DEVIATION OF POINTS ABOUT LINE IN PROBIT PLOT
C     WT      WEIGHT VALUE (NEED TO BE SUPPLIED BY USER ONLY IF USING
C             WEIGHTED LEAST SQUARES APPROACH
C     J       NUMBER OF DOSE LEVELS
C     K       NUMBER OF DATA POINTS PER DOSE LEVEL
C
C     INFORMATION WHICH MUST BE CONTAINED IN DATA DECK
C     PROBIT, AMW, PPM, ANAME, TIME, DAPHZA, J, K
```

```
C
C     ALL DATA READ IN IS WRITTEN OUT AGAIN IMMEDIATELY AFTERWARD FOR CHECKING
C
C
C     J = 0    CALL EXIT - PROGRAM TERMINATED (PLACE ZERO IN COLUMN 4
C              OF LAST CARD IN DATA DECK
C
      DIMENSION PROBIT(100),SNO(30),APP(30,30),ANAME(4,30),TIME(5),
     *DAPNDA(5),FIS>(5),PAGE(20,30),BP(30,30),FLCS8(30),AS(30),
     *SIGMAX(30),DUP*P(10,10),U(30),V(30),WT(30),P(30),YY(30),X(30,30),
     BY(30,30),WFLCS(30),WTSIGX(30),WT2(100),JOBSE(30),APP*(30)
      I = 6
      WRITE(61,206)
      DO 2 LK = 1,10
      READ(68,108) (DUMMY(KL,LK), KL = 1,10)
    2 WRITE(61,205) (DUMMY(KL,LK), KL = 1,10)
      I* = 0
      DO 3 LJ = 1,10


      DO 3 JI = 1,10
      I* = I* + 1
    3 PROBIT(I*) = DUMMY(I,LJ)
    1 I = I + 1
      READ(68,102) J, APP(I), (ANAME(II,I), II = 1,4)
      WRITE(61,200) J,APP(I), (ANAME(II,I), II = 1,4)
      WRITE(61,210)
      IF (J) 105,199,17
   18 JOBSE(I) = J
      DO 30 M = 1,4
      WRITE(61,211)
      READ(66,102) K, PP*(M,I)
      WRITE(61,211) K,PP*(M,I)
      READ(66,103) (TIME(K),DAPNDA(M),WT(M), M = 1,K)
      WRITE(61,202) (TIME(M),DAPNDA(M),WT(M), M = 1,K)
C
C     THIS STATEMENT CONVERTS PER-CENT SURVIVORS TO LOG PER-CENT SURVIVORS
```

```
      DO 20 K = 1,6
   20 FISH(K) = ALOG10(ALOG(1.0/(1.0-P(K))))
C
C     MASK = 40      SUBROUTINE PRINT OUT
C
C     MASK = 400000 NO SUBROUTINE PRINT OUT
C
      MASK = 40
      WRITE(61,104) (ANAME(II,I), II = 1,40)
C
C     THIS SUBROUTINE USES A WEIGHTED LEAST SQUARES.  THE WEIGHT FACTOR IS
C     1/(WT SQUARED) THEREFORE A SMALL NUMBER FOR WT RESULTS IN A LARGER
C     EMPHASIS BEING PLACED ON THAT DATA.
C
      CALL WEIGHT(K,TIME,FISH,WT,CONST,SLOPE,MASK,R,S)
C
C     THIS STATEMENT STORES THE STANDARD DEVIATION IN WT2 AND CAN BE USED
C     IN LATER CALCULATIONS AS A WEIGHT FACTOR
C
      WT2(M) = S
C
C     THE NEXT THREE STATEMENTS CALCULATE THE PROBIT VALUE TO BE USED
C     IN THE SEEDING PLOT.  A ROUND OFF COMMAND IS USED HERE, BECAUSE ONLY
C     PROBIT VALUES FOR WHOLE NUMBERS WHERE SUPPLIED INITIALLY
C
      YP = 40.0*SLOPE + CONST
      IP = IFIX(10.0**YP + 0.5)
      PROBIT(M,I) = PROBIT(IP)
C
C     LOG 1/WT IS CALCULATED AT THIS POINT
C
   30 SP(M,I) = ALOG10(ALOG(1.0/(P(M,I))**2))
      MASK = 40
      WRITE(61,107) (ANAME(II,I), II = 1,40)
C
C     THIS SUBROUTINE USES UNWEIGHTED LEAST SQUARES TREATMENT
C
      CALL LINEFIT(1,I,SP,PROB,A,B,SIGMA?,MASK)
```

```
      ELCSB(I) = (S - A)/B
      APM(I) = AMB(I)*10**(3 - ELCSB(I))
      SIGMA(I) = SIGMB/B
      DO 35 M = 1,J
      U(M) = GA(M,I)
   35 V(M) = PSB(M,I)
      *RSQ = 0.0
C
C     THIS SUBROUTINE USES WEIGHTED LEAST SQUARES TREATMENT
C
C     SAME DATA USED - THIS TIME WEIGHT FACTORS INCLUDED
C
C
      CALL WEIGHT(J,W,0.072,ACONST,B,SLOPE,*RSQ,R,SIGMB)
      *TLCSB(I) = (S - ACONST)/SLOPE
      *TSIGMA(I) = SIGMB/SLOPE
C
C     THE CORRELATION (R) OF UNWEIGHTED POINTS TO LINE ARE
C     CALCULATED AT THIS POINT IN THE PROGRAM
C
      SUMYSQ = 0.0
      SUMXSQ = 0.0
      SUMXY = 0.0
      SUMX = 0.0
      SUMY = 0.0
      DO 40 M = 1,J
      SUMX = GA(M,I) + SUMX
      SUMY = PSB(M,I) + SUMY
      SUMXY = GA(M,I)*PSB(M,I) + SUMXY
      SUMXSQ = GA(M,I)*GA(M,I) + SUMXSQ
   40 SUMYSQ = PSB(M,I)**PSB(M,I) + SUMYSQ
      SUSUMX = SUMX*SUMX
      SUSUMY = SUMY*SUMY
      SMXSMY = SUMX*SUMY
      SUMXMY = J*SUMXY
      SMYSQN = J*SUMYSQ
      SMYSQM = J*SUMYSQ
      RR(I)=(SUMXMY-SMXSMY)/SQRT((SMYSQN-SUSUMX)*(SMYSQM-SUSUMY))
```

```
      IEND = I
      GO TO 1
  190 WRITE(61,195)
      WRITE(61,185)
      DO 58 I = 1,IEND
   58 WRITE(61,186) (ANAME(II,I), II = 1,4), XDOSE(I),ELC50(I),APPM(I),
     $  BP(I), SIGPRB(I)
  180 FORMAT(10F5.3)
  181 FORMAT(I4,F10.4,A6)
  182 FORMAT(I4,F10.4)
  183 FORMAT(3F10.4)
  184 FORMAT(1H0,A80//)
  185 FORMAT(5X,14H COMPOUND NAME,6X,7H NO. OF,13X,5H LC50,10X,12H CORRE
     $LATION,3X,8H SIGMA/SQR,5X,5H DOSE,4X,8H LOG 1/C/25X,7H LEVELS,6X,8H
     $LOG 1/C,5X,4H PPM//)
  186 FORMAT(1H0,A8E,3X,I2,3X,5(5X,F7.3))
  187 FORMAT(1H0,A8E,15H PROBIT PLOT.../)
  188 FORMAT(1H1,63H CALCULATED LETHAL CONCENTRATION (LOG 1/C, C = MOLA
     $R CONCENTRATION)//4H FOR 50 PERCENT OF THE TEST ANIMALS AT 48 HOUR
     $S.)
  200 FORMAT(1H1,I4,F10.4,A6)
  201 FORMAT(1H0,I4,F10.4 /)
  202 FORMAT(1H ,3F10.4)
  205 FORMAT(1H0,10F5.3)
  206 FORMAT(1H1,14H PROBIT VALUES)
  210 FORMAT(1H ,3X,*********************************** /)
  211 FORMAT(1H0,5X,*****************)
      CALL EXIT
      END
      SUBROUTINE WEIGHT(N,U,V,AT,A,B,XBAR,R,SIGMA)
      DIMENSION U(30),V(30), WT(30), RN(30), YP(30)
      RN = FLOAT(N)
      SUMR = 0.0
      SUMY = 0.0
      SUMDELXY = 0.0
      SUMDELX2 = 0.0
      SDELRY = 0.0
      SDELA2 = 0.0
```

```
      SUMRSQ = 0.0
      SUMW = 0.0
      DO 1 I = 1,N
      W = 1.0/(WT(I)*WT(I))
      SUMW = SUMW + W
      SUMX = SUMX + W*U(I)
    1 SUMY = SUMY + W*V(I)
      XBAR = SUMX/SUMW
      YBAR = SUMY/SUMW
      DO 2 I = 1,N
      W = 1.0/(WT(I)*WT(I))
      DELX = W*(U(I) - XBAR)
      DELXY = (W*(V(I) - YBAR))*DELX
      DELX2 = DELX**2
      SUMDELXY = SUMDELXY + DELXY
    2 SUMDELX2 = SUMDELX2 + DELX2
      B = SUMDELXY/SUMDELX2
      A = YBAR - B*XBAR
      DO 3 I =1,N
      IF(ABS(U(I)).LT. .1E-05) GO TO 3
      DSELB2 = ((V(I) - A )/U(I) - B)**2 + SDELB2
    3 SDELA2 = (V(I) - B*U(I) - A)**2 + SDELA2
      DELTAB = SQRT(SDELB2/FN)
      DELTAA = SQRT(SDELA2/FN)
      DO 4 I = 1,N
      YY(I) = B*U(I) + A
       R(I) = V(I) - YY(I)
    4 SUMRSQ = SUMRSQ + R(I)*R(I)
      SIGMA = SQRT(SUMRSQ/FK)
      IF(MASK .EQ. 4HMASK)  RETURN
      WRITE(61,195) A, B(1), B(N)
      WRITE(61,99) B,A,DELTAB,DELTAA
      WRITE(61,185)
      WRITE(61,188) (U(I),V(I),YY(I),WT(I),R(I), I = 1,N)
      WRITE(61,181) SIGMA
      RETURN
```

```
 99 FORMAT(1H0,25HTHE BEST LINE FIT IS ...,2X,F10.6,8H * X  +  .
    +F12.6//32H THE UNCERTAINTY IN THE SLOPE IS,3X,F10.6,
    +24H AND IN THE INTERCEPT IS,3X,F10.6//)
100 FORMAT(5(4X,F10.6))
101 FORMAT(1H0,10X,10HSIGMA Y =  ,F10.6/)
105 FORMAT(1H ,I5,2X,29H POINTS AND X RUNNING FROM  ,F10.6,4H TO  .
    +F10.6)
106 FORMAT(1H ,7X,2H X,12X,2HY,12X,4HF(X),9X,4H  WT,9X,10HRESIDUALS )
    END
    SUBROUTINE LINEFIT(N,KK,X,Y,A,B,SIGMAY,MASK)
    DIMENSION X(30,99),Y(30,30)
    RN= FLOAT(N)
    SUMX= 0.0
    SUMY= 0.0
    SUMDELXY = 0.0
    SUMDELX2 = 0.0
    SDELR2= 0.0
    SDELA2= 0.0
    RESID2= 0.0
    DO 1 I=1,N
    SUMX = SUMX + X(I,KK)
  1 SUMY = SUMY + Y(I,KK)
    XBAR = SUMX/ RN
    YBAR = SUMY/ RN
    DO 2 I = 1,N
    DELX = X(I,KK) - XBAR
    DELXY = (Y(I,KK) - YBAR)*DELX
    DELX2 = DELX ** 2
    SUMDELXY = SUMDELXY + DELXY
  2 SUMDELX2 = SUMDELX2 + DELX2
    B = SUMDELXY / SUMDELX2
    A = YBAR - B * XBAR
```

```
      DO 3 I= 1,N
      IF(ABS(X(I,NN)).LT. .1E-35) GO TO 3
      SDEL42 =(Y(I,NN) - AD/X(I,NN) - BD )**2 + SDEL82
    3 SDELA2 =(Y(I,NN) - B*X(I,NN) - AD**2 + SDELA2
      DELTAB = SQRT(SDEL82 / ND)
      DELTAA = SQRT(SDELA2 / ND)
      IF ( MASK .EQ. -HMASK ) RETURN
      WRITE(61,105) N, X(1,NN), X(N,NN)
      WRITE(61,99) F,A,DELTAB,DELTAA
      PRINT 106
      DO 4 I= 1, N
      F = B*X(I,NN) + A
      RESID = Y(I,NN) - F
      WRITE(61,100) X(I,NN), Y(I,NN), F, RESID
    4 RESID2 = RESID ** 2 + RESID2
      SIGMAY = SQRT(RESID2/ ND)
      PRINT 101,SIGMAY
      RETURN
   99 FORMAT(1H0,26HTHE BEST LINE FIT IS ,.....,2X,F18.6,8H * X  +  .
     +F12.6//32H THE UNCERTAINTY IN THE SLOPE IS,3X,F18.6,
     +24H AND IN THE INTERCEPT IS,3X,F18.6//)
  100 FORMAT( 1H , 4(2X,E12.6))
  101 FORMAT(1H0,18X,18HSIGMAY =  ,F18.6//)
  105 FORMAT(1H ,I5,2X,28H POINTS AND X RUNNING FRO  ,F12.6,4H TO ,
     +F18.6)
  106 FORMAT( 7X, 2H X , 12X, 1HY, 12X, 4HF(X) , 6X,
     + 10HRESIDUALS )
      END
         FINIS
```